# STUDYING OF THE SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF N-ALKYL DERIVATIVES OF CHITOSAN BASED ON *APIS MELLIFERA*

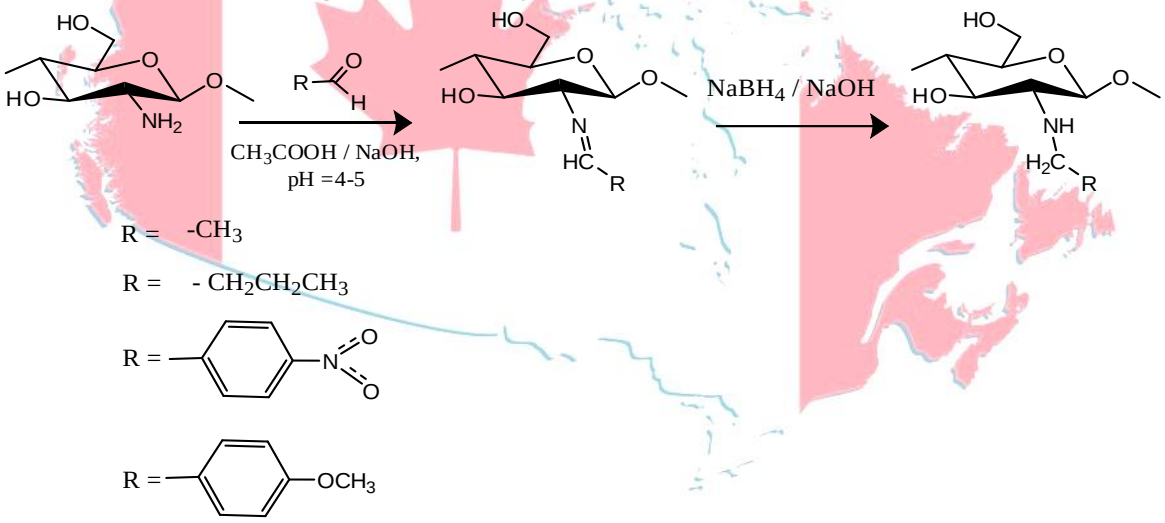
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***Abstract.*** *Recent studies have increasingly focused on the synthesis of chitosan and its novel derivatives, which exhibit a broad spectrum of biological activities, including antibacterial, antifungal, biocompatible, and biodegradable properties. These attributes significantly expand the potential applications of chitosan across various fields. The functional utility of chitosan is closely related to its solubility, which remains a key challenge. To address this, strategies to enhance solubility typically involve the introduction of alkyl groups into the chitosan backbone. In this context, our research involved the synthesis and comprehensive characterization of a series of N-alkylated chitosan derivatives, with the aim of improving their solubility and expanding their applicability.*

***Keywords:*** *Chitosan, N-alkylation, water solubility, Schiff bases, IR spectrum*

The interest in chitosan and its derivatives has been increasing due to their high biological activity against various viruses, fungi, and bacteria, as well as their low toxicity. These substances also retain important functional properties such as biodegradability and biocompatibility. Consequently, their applications are expanding across multiple fields, including medicine, food industry, agriculture, wastewater treatment, and environmental protection [1]. Chitosan is obtained through the deacetylation of chitin, a naturally occurring polymer found in the cell walls of various organisms such as insects, crustaceans, fish scales, plants, and fungi. Although chitin is a widespread natural polymer, its deacetylated form, chitosan, is relatively rare in nature. In 1954, Kreger first identified chitosan in the mycelium and sporangiophores of *Hycyomyces blakesleeanus*. Subsequently, chitosan has been detected in certain fungi and crustacean species. Bartnicki-Garcia and Nickerson reported that *Mucor rouxii* cell walls contain approximately 32.7% chitosan [2]. The primary factors influencing the biological activity of chitosan are closely related to its solubility properties. Chitosan dissolves in dilute organic solvents at pH values below 6.5, such as formate, acetate, pyroxene, as well as in 10% citric and lactic acid solutions. This solubility behavior is primarily attributed to the pKa value of the N-amino groups of

chitosan, which is approximately 6.5. The limited solubility of chitosan in aqueous solutions with pH ≥ 6.5 presents constraints for its application in various fields, including cosmetics, the food industry, and biomedicine. Consequently, extensive research has been conducted to enhance the water solubility of chitosan. Since the use of chemicals in biological applications necessitates material treatment at neutral pH, developing water-soluble derivatives of chitosan is crucial for expanding its utility as a biofunctional material. Enhancing the solubility of polymers can be achieved through chemical modification of N-amino functional groups. This approach leads to the formation of N-substituted derivatives that are soluble in aqueous media. Common N-substitution reactions include N-alkylation, N-acylation, and N-hydroxyacylation [3]. In our research, we synthesized N-alkyl derivatives of chitosan derived from Apis mellifera, a locally sourced raw material. The synthesis of chitosan N-alkyl derivatives involves the use of aldehydes or ketones, which react with chitosan to form Schiff bases through condensation reactions. These reactions are typically performed in a binary solvent mixture of acetic acid and methanol. Initially, the process occurs under homogeneous conditions. Over time, gelation ensues due to the insolubility of the formed Schiff bases. Subsequently, N-alkyl derivatives are synthesized through the reduction of these Schiff bases using reducing agents such as NaBH4, KBH4, or NaBH3CN. The following is the N-alkylation reaction of chitosan:




Scheme-1. N-alkylation reaction of chitosan with aldehydes

During our research, it was observed that the N-alkylation of chitosan with aldehydes, conducted in DMSO as the solvent at a temperature of 70ºC for 3 hours, yields products with high efficiency. The acceleration of the reaction in DMSO is attributed to reduced solvation of the remaining nucleophilic ions. Unlike aprotic solvents, protic solvents strongly solvate nucleophilic ions, thereby diminishing their reactivity. Based on these findings, N-alkyl derivatives of chitosan were synthesized using ethanal, butanal, p-nitrobenzaldehyde, and p-methoxybenzaldehyde. To

characterize the structures of the obtained products, IR spectroscopy was employed. The results are summarized in Table 1.

Table 1.

IR spectral regions of *Apis mellifera* chitosan and its N-alkylation products ($cm^{-1}$) [4]

| **Functional groups and vibrational types** | **Chitosan** | **Ethanal** | **Buthanal** | **p-methoxy benzaldehyde** | **p-nitro benzaldehyde** |
|---|---|---|---|---|---|
| υ O-H pyranose ring | 3353 | 3381.35 | 3359.4 | 3386.41 | 3356 |
| υ N-H pyranose ring | 3102.3-3260.2 | - | 3260 | 3277.40 | - |
| $\upsilon_{as}$ $CH_2OH$ contained ($CH_2$) | 2918 | 2927.35 | 2917 | 2918 | 2924.6 |
| $\upsilon_s$ pyranose ring and $CH_3$ contained (C-H) | 2872 | 2873.12 | 2848.8 | 2849 | 2848 |
| υ (C=O) $NHCOCH_3$ group (amide I bond) | 1649.3 | 1668.61 | 1633 | 1635.77 | 1654 |
| δ ($NH_2$) $NHCOCH_3$ group (amid II bond) | 1588.3 | - | 1585 | - | 1583 |
| $\delta_{as}$ ($CH_2$) $CH_2$ OH group and $CH_3$ contained | 1420.8 | 1447.52 | 1426 | 1404.91 | 1517 |
| $\delta_s$ ($CH_3$) $NHCOCH_3$ group and $CH_3$ contained | 1373.5 | 1371.95 | 1375 | 1338.82 | 1345.5 |
| δ (C-H) pyranose ring | 1317 | 1307.06 | 1308 | 1312.15 | 1310 |
| NHCO complex vibrations of the | 1257 | 1236.41 | 1254 | 1253.70 | 1254 |

| | | | | | |
|---|---|---|---|---|---|
| band (amid III bond) | | | | | |
| $\upsilon_s$ (C-O-C) (glycoside bond) | 1149.2 | 1149 | 1148 | 1169.84 | 1169 |
| $\upsilon_{as}$ (C-O-C) ((glycoside bond) | 1060.3 | 1057.93 | 1052 | 1050.01 | 1052 |
| $\upsilon$(C-O) secondary OH group | 1027 | 1026.48 | 1025 | 1017.81 | 1022.8 |
| $\delta$ $CH_3$ va $\upsilon$(C-O) primary OH group | 945.84 | - | 927 | 927.32 | 927 |
| $\upsilon$ CH pyranose ring | 892.47 | 894.73 | 894 | 892.69 | 836.16 |

Based on the results presented above, the IR spectra of the N-alkylation products exhibit a shift of the amide I band to lower wavenumbers. Additionally, there is a notable increase in the intensity of absorption signals around approximately 1375 $cm^{-1}$ and 1450 $cm^{-1}$, corresponding to the symmetric and asymmetric deformation vibrations of the $–CH_3$ groups. These spectral changes provide evidence that the alkylation process has occurred.

**References:**

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