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SYNTHESIS OF CHITOSAN N-ACYL DERIVATIVES BASED ON APIS MELLIFERA

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Abstract: Currently, due to their biocompatibility, biodegradability, non-toxicity, and numerous therapeutic properties (including antioxidant, high activity against microbes and fungi, tumor and cancer cells, analgesic, and, hemostatic effects), interest in chitin and chitosan polyaminosaccharides for use in the biomedical, pharmaceutical, cosmetology, and food industries is increasing. Solubility is important in the manifestation of the biological properties of chitosan. In our research, we aimed to synthesize several N-acyl derivatives of chitosan obtained from local raw material and to study their structure using the FTIR method.

Keywords: Apis mellifera, chitin, chitosan, N-acylation, FTIR spectroscopy.

INTRODUCTION

Interest in chitosan and its derivatives, which exhibit high biological activity against various viruses, fungi, and bacteria, have low toxicity, and retain important activities such as biodegradation and biocompatibility, is increasing day by day. Because such substances are important not only in medicine but also in the food industry, agriculture, wastewater treatment, and environmental protection [1]. Chitosan is a deacetylation product of chitin found in the cell walls of various insects, crustaceans, fish scales, plants, or fungi. Although chitin is a common natural polymer in nature, its deacetylated form chitosan is rare in nature. In 1954, Kreger first identified its occurrence in the mycelium and sporangia of Hycomyces Blakesleeanus. Later, the presence of chitosan was found in some fungi and crab species. Bartnicki-Garcia and Nickerson found 32.7% chitosan in Mucor rouxii cell walls [2].

The most important factors affecting the biological activity of chitosan are related to its solubility. Chitosan dissolves in dilute organic solvents with pH < 6.5 (formic, acetic, pyruvic, 10% citric, and lactic acid). The main reason for this is that the pKa value of N-amino groups of chitosan is equal to 6.5. The solubility of chitosan in acidic aqueous solutions with pH ≥ 6.5 causes limitations in the fields of use (cosmetics, food industry, and biomedicine). Therefore, many studies have been conducted on increasing the solubility of chitosan in water. The use of chemicals in biological applications requires treatment of the material at a neutral pH. Obtaining a water-soluble derivative of chitosan is important for the use of the polymer as a biofunctional material. Increasing the solubility property of the polymer includes chemical modification of N-amino functional groups. As a result, N-substituted derivatives with the property of dissolving in aqueous medium are obtained. Examples of N-substitution reactions are N-alkylation, N-acylation, and N-hydroxyacylation [3]. During our research, we synthesized N-acyl derivatives of chitosan obtained from local raw material Apis mellifera and used the FTIR method to study its structure.

RESULTS AND DISCUSSION

During our research, first chitin and chitosan were isolated from Apis mellifera, and the structure of the obtained substances was analyzed using the FTIR spectroscopy method.

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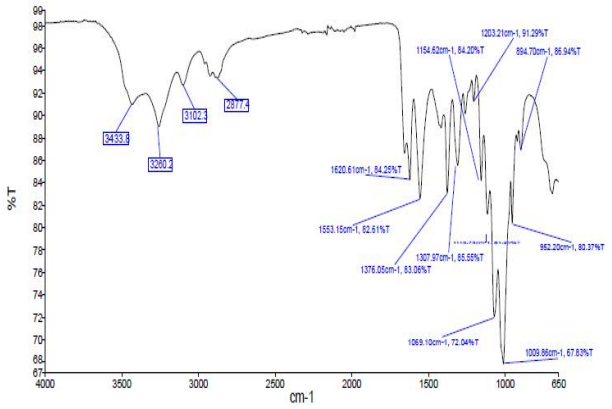

Fig.1. FTIR spectra of extracted chitin

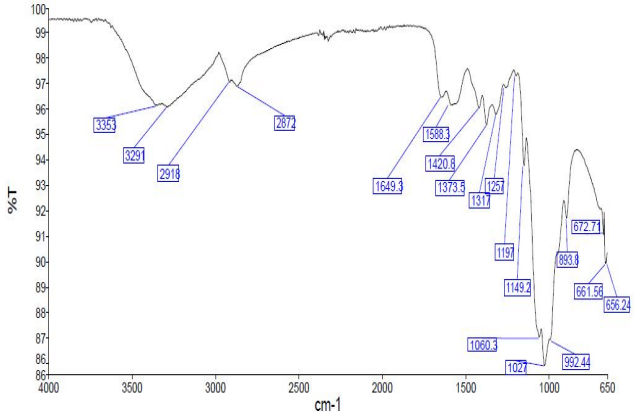

Fig.2. FTIR spectra of extracted chitosan

The IR spectrum of chitin obtained based on Apis mellifera is presented in Fig. 1, in which the amide I band of α-chitin consists of two peaks [4]. The fact that the amide I band of the extracted chitin consists of two peaks at 1652 and 1620 cm^{-1} indicates that the extracted chitin is in the α form. When studying the IR spectrum of chitin, the following signals were also observed: 3433 cm^{-1} (O-H valence), 3102-3260 cm^{-1} (N-H valence), 2877 cm^{-1} (methylene groups in the pyranose ring); 1553 cm^{-1} (amide II), 1430 cm^{-1} (CH_2 and CH_3 deformation), 1307 cm^{-1} (CH_2 wagging), 1154 cm^{-1} (asymmetric bridge oxygen stretching), 1115 cm^{-1} (asymmetric in phase ring stretching mode), 1069 cm^{-1} (saccharide rings), 1009 cm^{-1} (C–O asym. stretch in phase ring), 952 cm^{-1} (along chain) and 894 cm^{-1} (saccharide rings). Changes in signals indicate a decrease in polymer chain length and molecular mass [5,6].

The main changes in the IR spectrum of chitosan obtained by the deacetylation process occur in the areas of 1650 and 1590 cm^{-1}. The appearance and broadening of the band at 1590 cm^{-1} and the decrease in the intensity of the band at 1650 cm^{-1} indicate that the deacetylation process went well and chitosan was formed [5,7]. In the course of our study, the IR spectrum of extracted chitosan produced signals in the 1649 and 1588 cm^{-1} regions, and these changes are consistent with the data presented in the literature. Also, the change in the intensity of the absorption signals in the region of 900-1100 cm^{-1} indicates a decrease in the polymer chain length and molecular mass during deacetylation [5]. In the FTIR spectrum of chitosan extracted from Apis mellifera, signals were observed in the following areas: 3353, 3291, 2918, 2872, 1649, 1588, 1420, 1373, 1317, 1257, 1197, 1149, 1060, 1027, 992, 893, 672, 661 and 656 cm^{-1}.

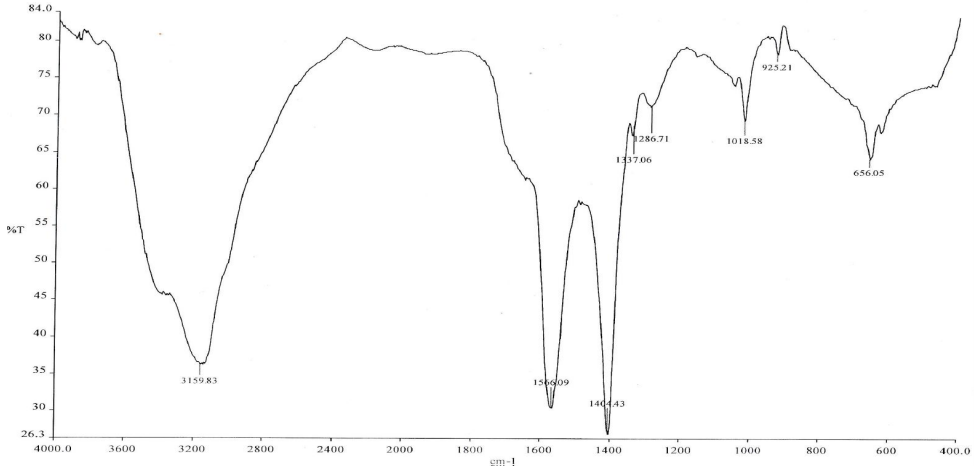

Fig.3. FTIR spectra of N-acyl chitosan

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When comparing the product of N-acylation of chitosan with acetic anhydride (Fig. 3) and the IR spectra of chitosan, an intense and narrower light absorption signal was formed in the region of 3159 cm^{-1}, which indicates that the amino groups have undergone a substitution reaction. The intense signals at 1566 and 1404 cm^{-1} are due to the asymmetric and symmetric valence vibration of the carboxylate (-COO-) anion and represent the process of N-acylation. Also, this change indicates the formation of intermolecular hydrogen bonds between the hydrophilic groups of the polymer chain and the carbonyl groups of the N-acyl functional group [8].

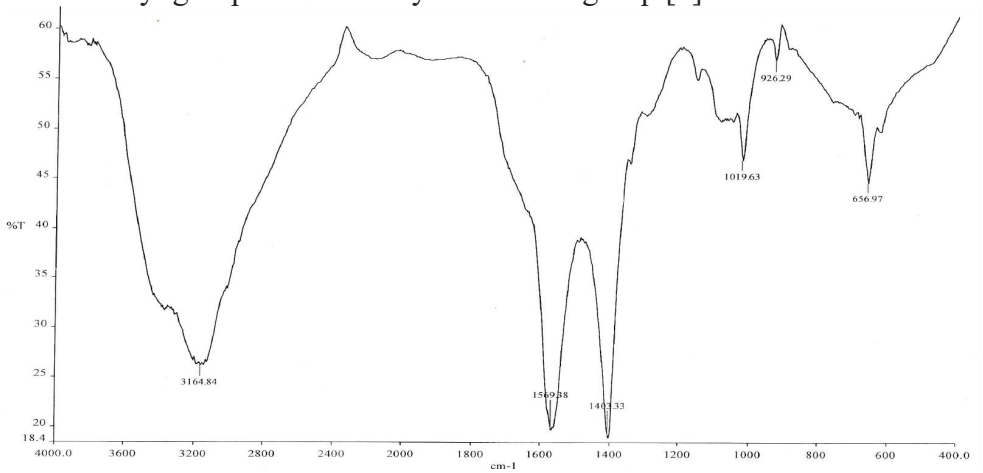

Fig.4. FTIR spectra of N-phthaloyl chitosan

The fact that the product of N-phthaloylation of chitosan mentioned above produced intense absorption signals in the IR spectrum at 3164, 1569, and 1403 cm^{-1}. It indicates that the N-phthaloylation process has taken place.

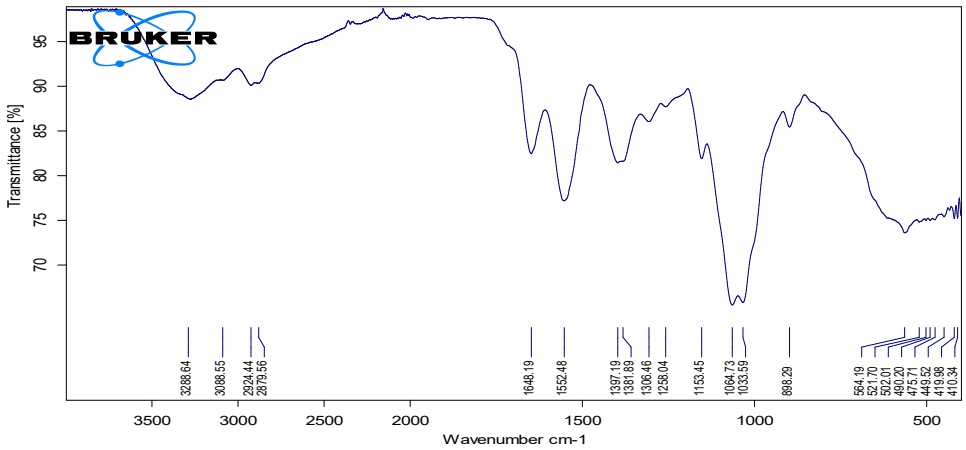

Fig.5. FTIR spectra of N-succinyl chitosan

In the IR spectrum of the product of N-succinization of chitosan with succinic anhydride mentioned above, the absorption signals caused by the valence vibration of –OH and –NH groups are narrower and shift to a lower wave number of 3288 cm^{-1} due to the introduction of succinyl groups. Also, due to N-succinylation, a shift of the absorption signals in the area of 2918 and 2872 cm^{-1} to 2924 and 2879 cm^{-1}, which occurred due to the valence vibration of the –CH groups in chitosan, was observed. A new absorption signal at 1397 cm^{-1} is observed due to the symmetric valence vibration of the –COO- group. Also, the intensity of the characteristic light absorption signal in the 1649 cm^{-1} region caused by the valence vibration of the C=O (amide I) group in the chitosan spectrum increased and moved to the 1648 cm^{-1} region, and the absorption signal in the 1588 cm^{-1} region caused

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by the deformation vibration of the amine
(-NH_2) functional group disappeared and a new absorption signal was formed in the 1562 cm^{-1} region. Indicates that amino groups are exchanged and -NH-CO- groups are formed [9].

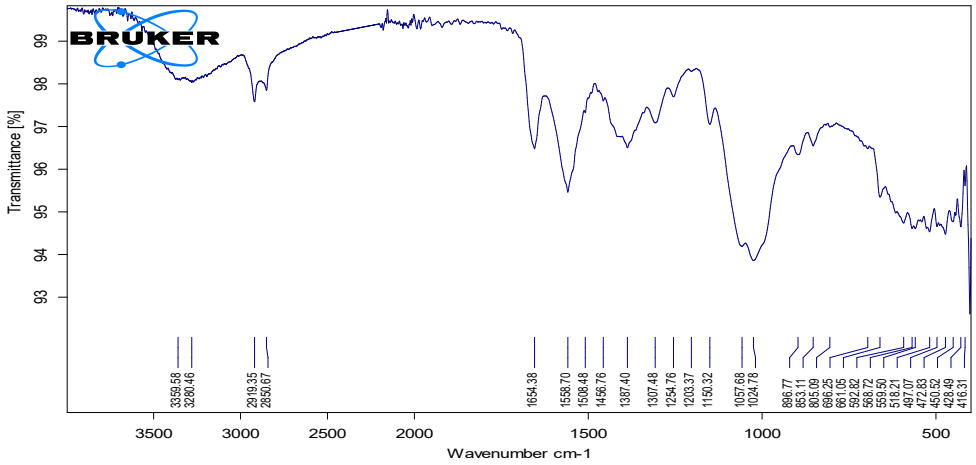

Fig.6. FTIR spectra of N-maleoyl chitosan

In the IR spectrum of the product of N-maleoylation of chitosan with maleic anhydride mentioned above, the absorption signals caused by the valence vibration of –OH and –NH groups are narrower and shift to lower wave numbers 3359 and 3280 cm^{-1} due to the introduction of maleoyl groups. Also, due to N-maleoylation, a slight increase in the intensity of the absorption signals in the region of 2918 and 2872 cm^{-1} and a shift to 2919 and 2850 cm^{-1} were observed. A new absorption signal at 1387 cm^{-1} is observed due to the symmetric valence vibration of the $–COO^-$ group. Also, the intensity of the characteristic light absorption signal in the 1649 cm^{-1} region caused by the valence vibration of the C=O (amide I) group in the chitosan spectrum increased and shifted to the 1654 cm^{-1} region, and the signal in the 1588 cm^{-1} region caused by the deformation vibration of the amine ($–NH_2$) functional group disappeared and a new absorption signal was formed in the 1558 cm^{-1} region. Shows that the exchange process of the groups took place and the formation of -NH-CO- groups.

MATERIALS AND METHODS.

Extraction of chitosan from Apis mellifera

The dead bees obtained were washed thoroughly with distilled water to remove impurities and dried in an oven to constant weight at a temperature of 40 ºC. Then 100 g of the crushed sample was taken for the extraction process. This dried material was soaked with an aqueous solution of 1N HCl (1:10 w/v) for 1 hour at 70-80 °C. The sample was then washed with distilled water until a relatively neutral pH was achieved and then the demineralized sample was dried to constant weight. Then, the demineralized samples were dried and weighed. They were then soaked in 1N NaOH (1:10 w/v) for 4 hours at 70-80 °C. After which the deproteinised sample was washed with distilled water until a neutral pH medium. For the decoloration process, the resulting material was soaked in a 30% H_2O_2 solution for 24 hours and washed with distilled water. After that, chitin obtained with a yield of 10% (10 g) was deacetylated in a 40% aqueous solution of NaOH (1:10 w/v) for 10 hours at 70-80 °C and then washed thoroughly with distilled water. The resulting light brown chitosan flakes were dried to constant weight with a yield of 6%.

Fourier Transform Infrared spectroscopy (FTIR)

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Chitin and chitosan obtained from Apis mellifera were characterized from 4000-650 cm^{-1} by a Perkin-Elmer FTIR spectrophotometer, N-acyl derivatives were characterized IRTracer-100 (SHIMADZU, 4000-400 cm^{-1}) and INVENIO S (BRUKER, 4000-400 cm^{-1}) FTIR spectrophotometer.

Synthesis of N-acyl derivatives of chitosan

0.1 g of chitosan was weighed on an analytical scale, dissolved in 20 ml of 2% acetic acid solution (pH = 3.8–3.9), and 10 ml of ethyl alcohol was added and stirred at room temperature for 1 hour on a magnetic stirrer. 10 drops (0.25 mmol) of acetic anhydride ($(CH_3CO)_2O$) were added to the resulting solution and stirred on a magnetic stirrer for 2 hours at room temperature. When a 12% solution of ammonia (NH_3) was added to the obtained solution until pH=8.5, a liquid-colored precipitate was formed. The resulting precipitate was washed in distilled water and filtered and dried at 60º C to constant weight with a yield of 73%. N-phthaloyl chitosan 71%, N-maleoyl chitosan 68.5%, and N-succinyl chitosan 67% were synthesized in the above method.

Conclusions

The structures of chitin, chitosan extracted on the basis of Apis mellifera and N-acyl derivatives synthesized on its basis were analyzed on the basis of FTIR spectroscopy, and the obtained results were found to be almost similar to the data presented in the literature.

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