Preparation of *Apis mellifera* carboxymethylchitosan and determination of its molecular weight and degree of acetylation

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Abstract. This article presents the results of obtaining biopolymers of carboxymethyl esters of chitosan from a new promising source - dry dead bees. Dried and crushed deadwood, collected during the spring renewal of the bee colony, was used. The process was carried out under general conditions according to the general method of alkylation. The properties of the resulting carboxymethylchitosans were studied by physicochemical analysis, their molecular weight by viscometry, the degree of mixing by conductometry.

1 Introduction

Chitosan and its derivative, carboxymethylchitosan, are the most common biologically active polymers. Due to their high biological activity, these biopolymers are actively introduced into various spheres of human activity [1]. One of the directions in obtaining promising drugs is chemical modification and the use of molecular fragments [2].

Studies on the synthesis of low-toxic biologically active substances in a number of O- and N-substituted derivatives of biopolymers and is devoted to the development of affordable preparative methods for the synthesis of such compounds, to identify the relationship between the structure and biological activity of substances with pronounced therapeutic properties. Many organic compounds potentially have pharmacological effects [3].

Carboxymethylchitosan CMCS is prepared by adding a carboxymethyl group to the chitosan structure. This modification increases its solubility in neutral and basic solutions without affecting other important characteristics. CMCS is obtained by carboxymethylation of hydroxyl and amine groups of chitosan [4]. Various substitutions of templates can be obtained depending on the reaction temperature used (Fig.-1). At room temperature, O-substitution is preferred, whereas at a higher temperature, N-substitution is the effective way. Taking into account the reaction conditions and reagents, various derivatives can be produced, i.e. N-, O-, N, O- or N, N-dicarboxymethyl chitosan [5].

Recently, there has been an increase in interest in chitosan and its derivatives. In addition, they have good biological activity, radiation resistance, the ability of film formation.

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These polymers fully meet the above requirements, since they undergo biological cleavage without the formation of harmful substances, are not deficient and are relatively inexpensive in relation to medical products [6].

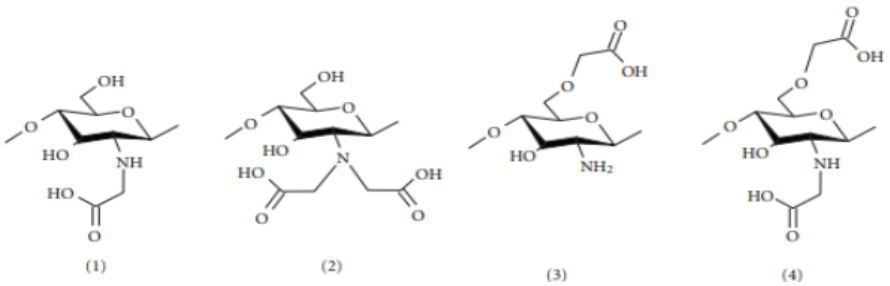


Fig. 1. Chemical structure of various types of carboxymethylchitosan (KMCS): (1) N-KMCS, (2) N,N- KMCS, (3) O- KMCS, and (3) N,O- KMCS (shows modification in the D-glucosamine link).

2 The experimental part

Based on the above, the production of chitin-chitosan and its derivatives is one of the most important issues, this gives us researchers the opportunity to consider honey bees, that is, bee subspecies as a new promising method for obtaining chitin and chitosan [7].

The strength of the bee family (the mass of worker bees in the bee family, measured in kg) is, on average, 7.5 -8 kg. In summer, during the period of active honey harvesting and in spring after wintering, the bee family is renewed by almost 60-80% [8]. The synthesis of CMS was carried out on the basis of the methodology presented in [9] literature.

Special attention should be paid to the fact that the samples after the chitosan carboxymethylation reaction are dissolved in water, whereas the original chitosan is insoluble in water. This is a consequence of the introduction of hydrophilic carboxymethyl groups, which once again confirms the fact of the formation of CMCS [10-11].

Based on experimental data on chitosan carboxymethylation, the following possible reaction mechanism is proposed [12]:

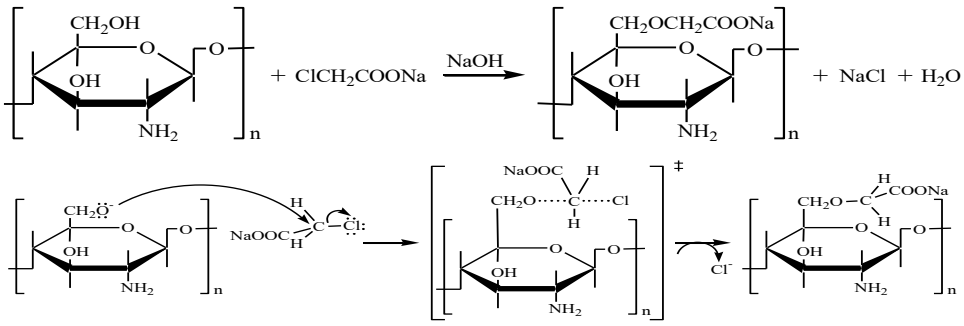


Fig. 2. Mechanism of reaction of synthesis of carboxymethylchitosan from chitosan

The reaction of chitosan with MHC in the presence of sodium hydroxide leads to the formation of the sodium salt of carboxymethyl chitosan. Since at pH >7 in an alkaline medium, the nucleophilicity of hydroxyl groups (OH) in the sixth carbon atom (C6) of the elementary units of the chitosan macromolecule is greater, and the hydroxyl group (C6) is spatially smallest. Consequently, in this case, the OH group in chitosan is replaced by a chlorine atom in monochloroacetic acid.

In our study, we measured 0.25 g of chitosan synthesized from a pod of Apis Mellifera bees collected and planted in the spring. Isopropyl alcohol was mixed with water in a ratio of 1:1, measured in a volume of 20 ml, placed on chitosan and stirred for 0.5 h in a magnetic stirrer at room temperature. Then the beaker was filled with 10 ml of 20% NaOH solution and stirred at 28 ° C for 1 hour. 0,28 g of monochloroacetic acid (MCA) was measured, slowly added to the glass and stirred at 65 °C for 2,5–3 hours.

The mixture was left for 8-9 hours. Then it was neutralized with 1.5 ml of glacial acetic acid, thoroughly washed with absolute alcohol and filtered on a Buchner funnel. After drying at room temperature, it was measured. We took 0.26 g of carboxymethylchitosan with a yield of 79%.

The carboxymethylchitosan obtained by this method from the chitosan of the bee podmore is an odorless, yellowish powdery substance [13].

Based on the analysis of the literature, a number of experimental stages of the chitosan carboxymethylation reaction Apis Mellifera have been developed:

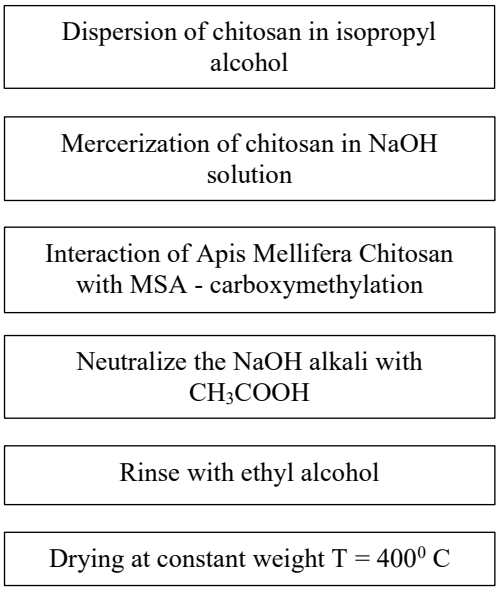


Fig. 3. Scheme of formation of carboxymethylchitosan from chitosan of bee podmore

3 Results and discussion

The degree of deacetylation of carboxymethyl chitosan was determined by conductometric titration. This method is based on the measurement of electrical conductivity, which is proportional to the concentration of electrolyte solutions. The advantage of this method is high sensitivity, sufficiently high accuracy (relative error of determination 0.01%), simplicity of methods, the possibility of studying colored and turbid solutions. The substitution of carboxymethyl groups in KMXZ samples was studied by conductometric titration in experiments conducted at Mettler-Toledo AG, Analytical CH-8603 Schwerzenbach, Switzerland. Solutions of 1 g/l of carboxymethylchitosan in 0.1 n. HCl solution and 0.5 n. NaOH solution for titration were prepared for conductometry.

At the same time, the change in electrical conductivity (Gsm) was controlled depending on the volume (V) of the titrant added to the solutions of samples prepared in 0.1 n HCl. At

the same time, the change in electrical conductivity was monitored depending on the volume of the titrant added to solutions prepared in 0.1 n HCl with 0.5 n NaOH [14].

The composition of functional groups (NH2 and NH2, COOH) in the samples of CMCS was calculated and the degree of substitution (NW) was determined.

The composition of NH-groups is determined by the following formula:

$$\omega_N = N_{mN}\Delta V_{N/g} \quad (1)$$

The composition of the CH2 COOH groups is determined by the following formula:

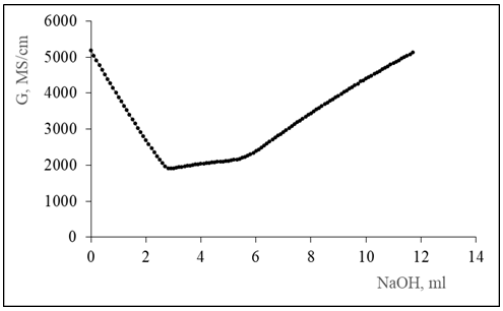
$$\omega_{karb} = N_{mkarb}\Delta V_{karb/g} \quad (2)$$

N is the normality of the alkali solution, ml and g-eq/l, respectively
N_{krab} - the number of carboxymethyl groups;
g - the mass of the polymer, g.

$$AD = 161 \times \frac{\omega_{karb}}{5900-59\times A} \quad (3)$$

where *A* is the general exchange degree 0.05 g of CMCS was dissolved in 25 ml of 0.02 n NaOH. After titration with 0.1 N NaOH solution, the electrical conductivity values were determined every 30 seconds. According to the graph, it is possible to determine the degree of substitution of the carboxyl group in the CMCS molecule from the amount of alkali consumed for titration.

The conductometric titration curve of carboxymethylchitosan, obtained at a temperature of 650C, a reaction time of 4 hours and a ratio of CS / MCA 1:1, is represented by a dashed line corresponding to a known range of titrant consumption. (fig.-2).



$$\chi = N_1 \times V_1 - N_2 \times V_2$$

$$\text{DA} = \frac{\chi}{\chi + \frac{m\times 0.9 - \chi\times 161}{203}}$$

Fig. 4. Conductometric titration line with CMCS solution.

where *V1* is the volume of acid, ml; *V2* is the volume of alkali required for titration, ml; *N1* is the normality of acid, mol-eq/ml; *N2* is the normality of alkali, mol-eq/ml; m is the mass of carboxymethylchitosan, mg.

At the initial stage of titration of CMCS with NaOH solution, the interval from 0 to V1 corresponds to the volume of the base added to neutralize the strong acid (H3O+) present in the solution (Fig.-2). Further, characteristic segments (V1-V2) are observed, which correspond to the titration of carboxymethyl groups (CH2COH). Necessary for neutralization (NH3+; +NH2R; +NHR2; where R is CH2COH) titration, a small segment (V2–V3) corresponding to the volume of the base (NaOH) is observed. With subsequent titration, an increase in the value of Gsm electrical conductivity is observed, characterizing an excess of a strong electrolyte (NaOH) [15]

Determination of molecular weights of CMCS by characteristic viscosity. The difference in the structure of chitosan and carboxymethylchitosan polymer chains is also invisible in the values of their characteristic viscosity values. A simple and effective method of viscometry

was used to determine the viscosity characteristics of chitosan and carboxymethylchitine solutions depending on the concentration of polymers (C). Solvent (water) release time t0=76.6 sec. Since chitosan has a polyelectrolytic effect, sodium acetate was added to the solution. For samples of carboxymethyl chitosan, sodium chloride was added to the solution to reduce the effect of polyelectrolyte. At least 3 measurements were carried out for each dilution of solutions. Calculations were carried out according to the Huggins equation [16].

$$\eta_{con}/_{S} \approx [\eta] + \kappa[\eta]2C \quad (4)$$

here $\eta_{con} = \eta_{rel}$ – specific viscosity,

$\eta_{rel} \approx {}^{t_1}/_{t_2}$ is the relative viscosity (where t_1 -is the solution exit time, t_2-is the solvent exit time); κ- is the Huggins coefficient; $[\eta]$-is the specific viscosity of the solution. $\eta_{con}/_{S}$ - is determined by extrapolating C→ 0 from the dependence C and is used to calculate the relative molecular weight (M_η) of the polymer - the Mark-Kuhn-Hauvink equation;

$$M_{\eta} \approx \frac{\left([\eta]/_{K}\right)1}{\alpha} \quad (5)$$

Here K = 1.4×10-4 and α = 0.83 are coefficients obtained from the literature for chitosan samples and K ≈ 7.92*10-4 dl/g, α = 1.00 for CMHC samples [17].

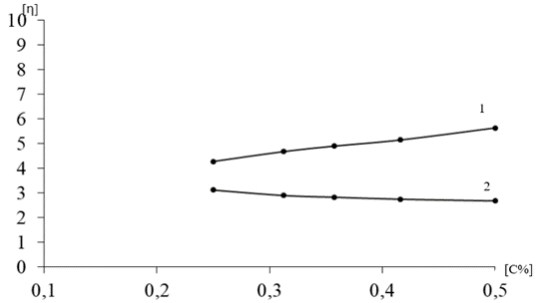


Fig. 5. The characteristic viscosity of CMHS: 1-relative viscosity. 2-conditional viscosity.

From the graph, it was determined that the characteristic [n] viscosity is equal to 3.7.

To calculate the molecular weight of carboxymethylchitosan, calculations were carried out using the Mark-Kuhn-Hauvink equation [η] ≈ KM η α. M η ≈ 180000 – 180000 for chitosan with CH3COOH+2% CH3COOH (K ≈ 1.44*10-4 dl/g, α = 0.83) in 2% solution, for carboxymethylchitosan M η ≈ 46700, H2O +2% NaCl (K ≈ 7.92*10-4 dl/g, α = 1.00). Such a difference in molecular weights indicates that the carboxymethylation of chitosan proceeds with a significant decrease in the molecular weights of the chains [18].

4 Conclusion

For the first time, O-carboxymethylchitosan was synthesized from Apis mellifera chitosan and its optimal conditions for its production were determined. The degree of carboxymethylchitosan exchange was determined by conductometry. During the carboxymethylation process according to our proposed method, samples with a degree of substitution of 82-91% were obtained. for the first time, the molecular weight of carboxymethylchitosan obtained from bee chitosan was calculated by the viscometric method.

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