OBTAINING CARBOXYMETHYLCHITOSAN ON THE BASIS OF BEES PODMOR AND DETERMINATION OF THE DEGREE OF DESACETYLATION (SD) BY THE METHOD OF CONDUCTOMETRIC TITRATION

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Abstract: This article presents the preparation of carboxymethylchitosan from a new promising source - dry dead bee chitosan. The degree of deacetylation of carboxymethylchitosan was studied and determined by conductometric titration.

Key words: dead bee, chitosan, carboxymethylchitosan, conductometry.

It is known that chitosan (CS) and its derivatives are widely used in the field of medicine, agriculture, etc. Chitosan derivatives, in particular, carboxymethyl chitosan (CMCS), which has a high biological activity and pronounced antibacterial and anticoagulant properties, attract particular attention of researchers [1]. CMCS possessing minimal toxicity and stability is widely used in almost all areas, such as medicine, food industry, agriculture, nuclear energy and textile industry [2].

The identification of such characteristics determines the comparative study of the structural morphology of the chitosan and CMC samples. In this regard, water-soluble samples of CMCS were synthesized and investigated by conductometric analysis [3].

In terms of prevalence in nature, chitosan is second only to cellulose and is obtained from products that are completely regenerated in nature. Chitosan is widely used in medicine, agriculture, textile industry, fabric dyeing and floral printing [4].

In the world, much attention is paid to chitosan derivatives obtained by chemical, physical or enzymatic modification of chitosan [5-6].

In connection with the widespread use of water-soluble derivatives of the chemical modification of chitin (CT), the synthesis of carboxymethylchitin (CMCS) is of great interest, a feature of which is that this polymer can be equally useful and non-toxic both for humans and for the environment. Of particular importance is the development of the most effective methods for modifying CMCS for the development of approaches and methods for the preparation of water-soluble derivatives of CT, which contain both the elements of the parent structure and new functional groups [7-8].

Today, the development of experimental methods makes it possible to obtain water-soluble, hydrophilic, biologically active, environmentally friendly, harmless and other drugs with special properties. One of the most important tasks is to obtain samples of carboxymethylchitosan (CMCS) with different levels of deacetylation and water solubility based on chitosan on a global scale, as well as to expand their application and scope [9].

Recently, we obtained chitosan from the dead bees Apis *Mellifera* and determined the chemical composition of the natural dry dead bees [10].

In our study, we measured 0,25 g of chitosan synthesized from dead *Apis Mellifera* bees collected and dried in spring. Isopropyl alcohol was mixed with water in a 1: 1 ratio,

measured in a volume of 20 ml, placed on chitosan, and stirred for 0,5 h in a magnetic stirrer at room temperature. Then a beaker was filled with 10 ml of 20% NaOH solution and stirred at 28^0 C for 1 hour. Measured 0.28 g of monochloroacetic acid (MCA), slowly adding to the glass and stirring 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, washed thoroughly with absolute alcohol and filtered on a Buchner funnel. After drying at room temperature, was measured. Took 0.26 g of carboxymethylchitosan with a yield of 79%.

Carboxymethylchitosan, obtained by this method from the chitosan of the dead bees, is odorless, yellowish powdery substance.

The degree of deacetylation of carboxymethylchitosan was determined by conductometric titration. Took an analytical weighed sample of carboxymethylchitosan 0,05 g. A standard solution of 0,1 M HCl was prepared from a fixed channel. The normality of the alkali solution was determined using a standard solution of hydrochloric acid. The sample was transferred to a beaker and dissolved in 0,1 N 25 ml HCl. To plot the titration curve, 100 µl of pre-titrated alkali (0,1 N NaOH) was added every 30 seconds.

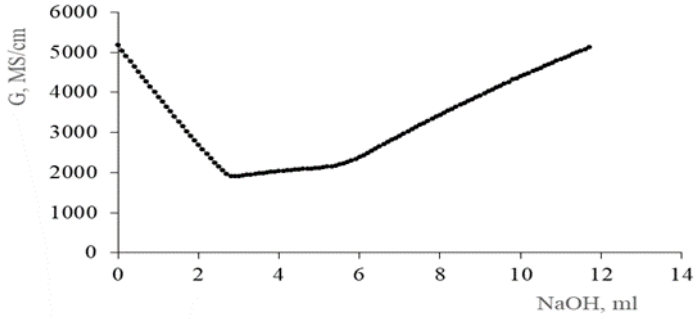

Figure. Conductometric titration curves solution CMCS.

The conductometric titration curve of carboxymethylchitosan obtained at a temperature of 65 ° C, a reaction time of 4 hours and a CS / CMCT ratio of 1: 1 is described as a dashed line corresponding to a certain range of titrant consumption (Figure).

$$x = N1 \cdot V1 - N2 \cdot V2,$$

$$СД = \frac{x}{x + \frac{m \cdot 0.9 - x \cdot 161}{203}} \cdot 100\%,$$

where V_1 is the volume of acid, ml; V_2 is the volume of alkali required for titration, ml; N_1 - acid normality, mol-eq / ml; N_2 - alkali normality, mol eq / ml; m is the mass of carboxymethylchitosan, mg.

At the initial stage of titration of CMCT with NaOH solution, the range from 0 to V_1 corresponds to the volume of the base added to neutralize the strong acid (H_3O^+) present in the solution (Figure1). Further, characteristic segments (V_1-V_2) are observed, which correspond to the titration of carboxymethyl groups (CH_2COOH). The titration required for neutralization (NH_3^+; + NH_2R; + NHR_2; where R – CH_2COOH) corresponds to the volume of the base (NaOH). During subsequent titration, an increase in the electrical conductivity Gsm is observed, which characterizes the excess of a strong electrolyte (NaOH) [11].

During the carboxymethylation of chitosan, the concentration of the alkali solution, temperature, duration of the carboxymethylation reaction and the amount of monochloroacetic acid (MCAA) were regulated by choosing the XS:MCAA ratio.

The effect of temperature (45-75°C) on the carboxymethylation reaction of *Apis Mellifera* chitosan was studied, since the possibility of obtaining KMCS samples with different contents of carboxymethyl groups is associated with a change in temperature. With increasing temperature, the SD of the samples increased from 80 to 91.7 (Table 1).

Table 1

The influence of temperature on the degree of substitution of the molar ratio of chitosan: MCCA (1:1), τ = 3 hours, the initial mass of chitosan is 0.25 g.

№	Sample	T⁰C	N %	ω(CH_2COOH)	SD %	P* %	outcome
1	KMCS -1	45	6,25	18,20	80	81	80
2	KMCS -2	55	5,40	20,25	91	85	82
3	KMCS -3	65	4,75	22,00	91,5	90	90
4	KMCS -4	75	4,50	21,05	91,7	88	88

ω*- percentage CH_2COOH, P** - solubility

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Conclusion

For the first time, O-carboxymethyl chitosan was synthesized from *Apis mellifera* chitosan and its optimal conditions for its production were determined, the kinetic parameters of the carboxymethylation reaction of dead bee O-carboxymethyl chitosan were determined, the concentration of sodium hydroxide was determined, which is 30%, and the required temperature for the reaction was 65⁰C. the duration of the polymer formation reaction process takes. The level of sample exchange was determined to be up to 80-96% by conductometric titration.

Literature:

1.Албулов А.И., Комаров Б.А., Самуйленко А.Я. и др. // Материалы В конференции "Но¬вые перспективы в исследовании хитина и хитозана". М.: ВНИРО, 1999. С.7-8.

2.Саттарова Д.М., Саттаров Т.А., Кодирханов М.Р., Рашидова С.Ш. "Перспективы формования нановолокон о-карбоксиметилхитозана bombyx mori для медицинского предназначения».Научный вестник НамГУ. 2020 йил 10-сон. С.102-108

3.Кличева О.Б., Рашидова С.Ш. "Синтез карбоксиметилированного хитина Bombix mory".- Конференция молодых ученных Актуальные проблемы химии природных соединений. Ташкент, 2015.- С.119

4.Kurbonova F.N., Ikhtiyarova G.A. Obtaination of carboxymethyl chitosan from inanimate bees and study of its properties by conductometry, UV-spectroscopy. Academician An International Multidisciplinary Research Journal.Vol.11, Issue 10, October 2021. P.1531-1535.

5.Под.ред. К.Г.Скрябина, Г.А.Вихаревой, В.П.Варламова"Хитин и хитозан: получение, свойства и применение". М.: Наука, 2002. –368с.
6.Рашидова С.Ш., Милушева Р.Ю. Хитин и хитозан Bombyx mori: синтез, свойства и применение. Ташкент. Фан, 2009. – 246с.
7.В.А. Александрова, Л.Н. Широкова. «Современные перспективы в исследовании хитина и хитозана» Мат. XI Междунар. конф. 2014. С.17-21
8.Chen R.H., Hwa H. D. Effect of Molecular-Weight of Chitosan with the Same Degree of Deacetylation on the Thermal, Mechanical, and Permeability Properties of the Prepared Membrane // Carbohydr. Polymer 1996. V. 29. №4
9.Ихтиярова Г.А., Курбанова Ф.Н. Apis mellifera жонсиз асалари хитозани асосида карбоксиметилхитозан синтези ва унинг таҳлили // НамДУ илмий ахборотномаси - Научный вестник НамГУ - №.11. 2021. Б. 92-95.
10.Feruza Kurbanova, Mahfuza Djumaeva, Gulbahor Khudoynazarova Preparation of Apis mellifera carboxymethylchitosan and determination of its molecular weight and degree of acetylation. Web of Conferences 460, 10029 (2023). tps://doi.org/10.1051/e3sconf/202346010029
11. Кличова О.Б. Автореферат дис. "О-карбоксиметилхитозан синтези, хоссалари ва қўлланиши"- Тошкент – 2017.С.14.
12.Kurbonova F.N., Ikhtiyarova G.A. Method of obtaining and physico-chemical properties of carboxymethyl ethers of chitosan from bees inanimate. Eurasian journal of academic research volume 2, issue 2 february 2022. P.467-472.