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STUDY OF THE PHYSICAL-CHEMICAL PROPERTIES OF CHITOSAN SYNTHESIZED BY THE CRYOGENIC METHOD FROM APIS MELLIFERA BEES

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Abstract: *Chitosan was synthesized from Apis Mellifera dead bees by traditional and cryogenic methods, and the degree of deacetylation, viscosity, and molecular mass of the synthesized sample was studied. Chitosan was synthesized by deacetylation of chitin under cryogenic conditions. NaOH concentration 40%, temperature 90 ± 3 °C, time 120 min. The degree of deacetylation, and molecular mass was determined using an Ubbelode viscometer. DDA was determined by conductometric titration under standard conditions. The degree of deacetylation of Apis Mellifera chitosan was 87%. synthesized by cryogenic method DDA of chitosan was 83% synthesized by traditional method. Conducting the synthesis in liquid nitrogen atmosphere leads to a certain decrease in the molecular mass of chitosan. This research aims to study the important parameters of chitosan molecule such as deacetylation level, viscosity and molecular mass.*

Keywords: *cryogenic method, chitin, chitosan, liquid nitrogen, viscosity, degree of deacetylation, molecular mass.*

Annotatsiya: *Xitosan jonsiz Apis Mellifera asalarilardan an'anaviy va kriogen usullar yordamida sintez qilindi, sintez qilingan namunaning deasetillanish darajasi, yopishqoqligi va molekulyar og'irligi o'rganildi. Xitozan kriogen sharoitda xitinni deatsetillash orqali sintez qilingan. NaOH konsentratsiyasi 40%, harorat 90 ± 3 °C, vaqt 120 min. Deatsetillanish darajasi va molekulyar og'irlik Ubbelohde viskozimetri yordamida aniqlandi. DAD standart sharoitlarda konduktometrik titrlash orqali aniqlandi. Kriogen usulda sintez qilingan Apis Mellifera xitozanining deatsetillanish darajasi 87% ni tashkil etdi. An'anaviy usul bilan sintez qilingan xitozanning DAD 83% ni tashkil etdi. Suyuq azot atmosferasida sintezning olib borilishi xitozanning molekulyar massasi ma'lum darajada pasayishiga olib keladi. Ushbu tadqiqot, xitozan molekulasining deatsetillanish darajasi, qovushqoqligi va molekulyar massasi kabi muhim parametrlarini o'rganishga qaratilgan*

Tayanch iboralar: *kriogen usul, xitin, xitozan, suyuq azot, yopishqoqlik, deatsetillanish darajasi, molekulyar og'irlik.*

Аннотация: *Хитозан синтезирован из подмора пчел Apis Mellifera традиционным и криогенным методами, изучены степень деацетилирования, вязкость и молекулярная масса синтезированного образца. Хитозан синтезировали деацетилированием хитина в криогенных условиях. концентрация NaOH 40%, температура 90 ± 3 °C, время 120 мин. Степень деацетилирования, молекулярную массу определяли с помощью вискозиметра Уббелоде. СДА определяли кондуктометрическим титрованием в стандартных условиях. Степень деацетилирования хитозана Apis Mellifera, синтезированного криогенным методом, составила 87%. СДА хитозана, синтезированного традиционным методом, составила 83%. Проведение синтеза в атмосфере жидкого азота приводит к некоторому уменьшению молекулярной массы хитозана. Целью данного исследования является изучение важных параметров молекулы хитозана, таких как уровень деацетилирования, вязкость и молекулярная масса.*

Ключевые слова: *криогенный метод, хитин, хитозан, жидкий азот, вязкость, степень деацетилирования, молекулярная масса.*

Introduction

Historically, the processing of natural products into biodegradable products has been an area of great interest in the medical, commercial, and scientific communities worldwide [1]. Chitosan belongs to the class of monosaccharides and is a cationic natural biopolymer. Recent scientific studies indicate its

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unique properties and excellent chemical and biological properties, such as biocompatibility, biodegradability, and antimicrobial properties, and its promising applications in human and veterinary, pharmaceutical, agro, and food industries [2]. Chitosan is easily obtained from natural sources such as crabs, shrimps, mulberry silkworms, dead bees, and mushrooms. and changes it from a solid state to a compact structure [4-6]. Due to this, water solubility and swelling properties increase [7-8]. As a result of the deacetylation of chitin, a copolymer of N-acetyl-glucosamines and glucosamines is formed. When this copolymer contains more than 50% glucosamine units, it is usually called chitosan.

Due to the presence of highly active amino groups in chitosan polymer, it is used in food [9-10], textile [11], cosmetic [12], environmental [13], biomedical [14-17] and pharmaceutical [18]] are widely used in the fields.

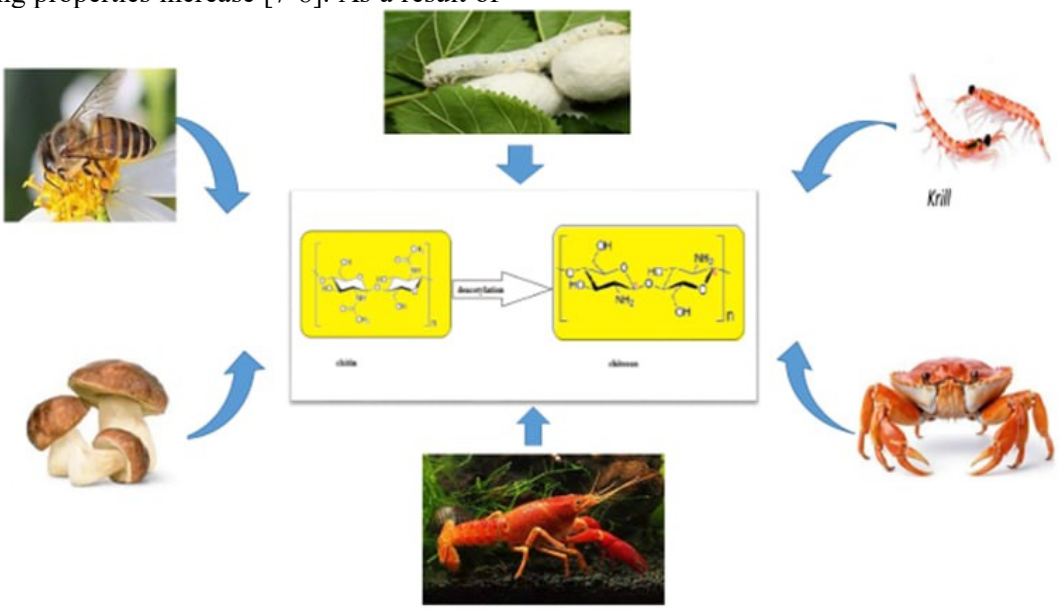


Fig.1. Natural sources and structural formulas of chitin and chitosan

Physico-chemical and biological activity of chitosan samples of different molecular masses was studied in the following works. Molecular mass (MM) affects the solubility of chitosan, its mechanical strength, and the elasticity of films and fibers, which determines the requirements for MM in various areas of chitosan application. The high MM of chitosan reduces its solubility in aqueous solutions and increases the tensile strength and moisture adsorption properties of the films [19-20]. Chitosans with high MM are used in medicine in the production of dental materials, in the treatment of burns and infections, in the fight against pests and plant diseases in agriculture, in the production of food products, in the production of films and microencapsulation in biotechnology [21 -22]. Chitosan samples at low MM with high DDA have better solubility and higher biological activity than samples at high MM. It has been studied that samples of chitin and chitosan with low MM and high DDA are more effective in fighting against wound healing and wound-causing microorganisms.

Research Object and Methods

Viscometry- (determination of viscosity properties) in determining the viscosity properties of chitosan solutions depending on the polymer concentration (C), with the flow time 106 seconds, in the presence of a buffer solvent of 0.66 molar sodium acetate salt in 2% acetic acid, is considered a simple and effective method was used [26].

Since chitosan macromolecules have polyelectrolytic properties in solution, sodium acetate salts were added to the polymer solution to prevent the polyelectrolytic effect. At least 5 measurements were made during each dilution by taking the point of 5-fold dilution of the solutions. The molecular mass of chitosan was determined using the viscometric method. For viscosity determination, 0.2 g of chitosan was dissolved in 0.33 M acetate buffer.

Calculations were made using the following equation:

$$M=[\eta]^{\alpha}/K$$

The characteristic viscosity of the samples was measured in an Ubbelode viscometer at a temperature of 25° C based on the solvent flow time in a thermostat. In this case, indicators a and K will be equal to α =083; $K=1{,}4\cdot10^{-4}$.

Experimentally, the intrinsic viscosity was determined by two-way graphical extrapolation of η_{ud}/s and $\ln(\eta_{otn}/C$ values to zero concentration. , which are described by equations [23].

Huggins equation: $\eta_{уд}/c=[\eta]+K_x[\eta]^2\times C$. ($K_x=tg\alpha/[\eta]^2$)

Kremer's equation: $\ln(\eta_{отн}/c)=[\eta]-K_{кр}[\eta]^2\times C$. ($K_{кр}=tg\beta/[\eta]^2$)

The quantities K_x and K_{kr} are related to $K_x+K_{kr}\approx 1/2$.

The dynamic viscosity of cryogenic and conventionally synthesized chitosan solutions was determined by Viscotester 2 plus rotational viscometer.

Conductometric titration. The degree of deacetylation (DDA) of chitosan was determined by conductometric titration on a Mettler-Toledo AG, Analytical CH-8603 (Schwarzenbach, Switzerland). The value of DDA is calculated according to the following formula:

$$NH_2=\frac{[Cm\ (NaOH)]\ (V_2-V_1)\times Mr}{m}$$

where: m is the mass of chitosan; M_r - a molecular mass of chitosan elementary unit; $\Delta V-$ is the volume of titrant NaOH, ml; C_m molarity of NaOH

Results and Discussion

Synthesis. To synthesize chitosan by cryogenic method, we use local raw material Apis mellifera dead bees, liquid nitrogen, HCl, and NaOH reagents.

Apis mellifera dead bees were ground in a porcelain mortar in the presence of 4% HCl acid in liquid nitrogen, then the mass was filtered and neutralized in distilled water. The resulting demineralized mass was dried at room temperature. For the deproteinization stage, it was carried out from a 4% solution of alkali solution at 80°C for 1 hour. After the reaction, the mixture was filtered and washed with distilled water and dried at 25°C. Then, to clean the dry mass from melanin pigment, it was left in a 3% solution of hydrogen peroxide for 24 hours at room temperature. The mixture was filtered and washed with distilled water and dried in a desiccator. The dry mass was subjected to the next deacetylation process in a 40% alkali solution at 90 ± 1°C for 120 minutes to synthesize chitosan [24-25]. After the deacetylation process, the product was filtered and neutralized in distilled water until pH=7, then washed in 96% ethyl alcohol. The resulting chitosan is dried in a lyophilic dryer.

By controlling the reaction time, chitosan with high DDA can be synthesized. When the reaction time is increased to 48 hours, the DDA can be up to 95%, but due to the degradation of the polymer in this reaction, the molecular weight of deacetylated chitin may decrease even to the monomer state [26-29].

Conductometric titration

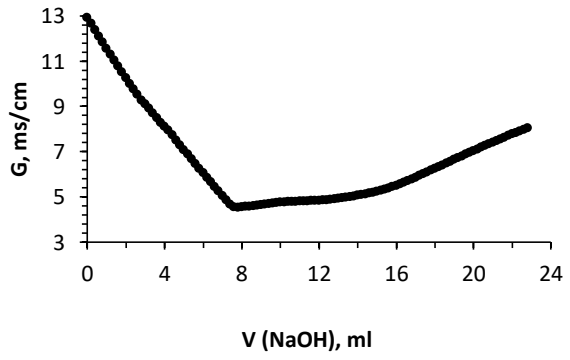
Currently, there are several methods used for the deacetylation of chitosan, of which Conductometric titration, i.e. alkali treatment, is widely used. In this method, sodium hydroxide is usually used for the deacetylation process. Factors affecting the degree of deacetylation are NaOH concentration, reaction time, temperature, chitosan concentration, and molecular weight of the starting polymer.

An acidic solution of chitosan is titrated with dilute NaOH and its electrical conductivity is measured. When the volume of added NaOH resists conduction, three different phases can be represented by curves and they can fit straight lines. In the first region of the curve, the conductivity decreases because NaOH causes the HCl in the chitosan solution to neutralize the H^+ (the mobility of H^+ is higher than that of Na^+). In the second region, the conductivity increases due to the neutralization of the –NH2 groups in the chitosan solution because the added Na^+ ions have a higher mobility compared to chitosan. Finally, in the third region, the permeability increases due to the increase in the concentration of Na^+ and OH^- ions.

Determination of deacetylation level of cryogenically and conventionally synthesized chitosan from *Apis mellifera* bees using a conductometer.

Titration was carried out by adding 35 ml of water to 15 ml of 0.97% solution of *Apis mellifera* chitosan (dissolved in 0.1 N HCl) and pouring 0.2 ml of 0.1 N NaOH solution into the resulting solution. First, 50 ml of the prepared solution was placed in the flask and magnetically stirred (Fig. 2(a)).

a)



b)

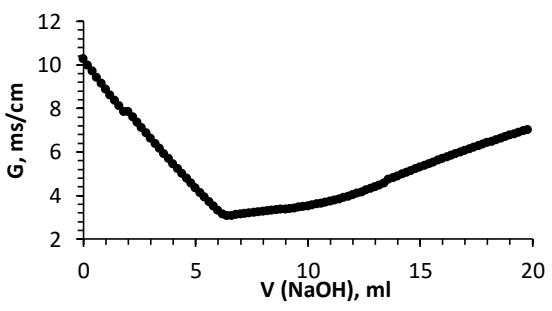


Fig.2. Conductometric titration curves of Apis Mellifera chitosan obtained by cryogenic (a) and conventional (b) methods

a) $\%DDA=\frac{[\Delta V]*CN*161\ ]}{m*1000}$

ΔV=15.2-7.4=7.8ml NaOH

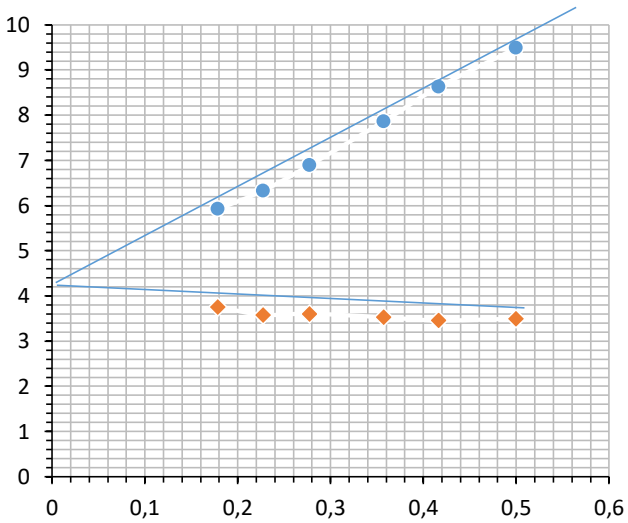
$\%DDA=\frac{[7.8]*0.1*161\ ]}{0.145*1000}=86.6\%$

To determine the DDA of chitosan in a traditional method, 35 ml of water was added to 15 ml of a 0.91% solution (dissolved in 0.1 N HCl) and the resulting solution was titrated with a 0.1 N NaOH solution. First, 50 ml of the prepared solution was placed in the flask and 0.2 ml of NaOH solution was added while rotating on a magnetic stirrer. (Figure 2(b)).

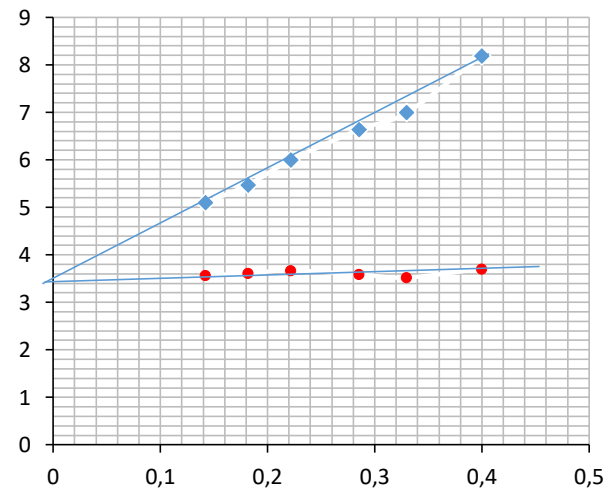
b) %DDA=[ΔV]*CN*161/(m*1000)

ΔV=13.4-6.4=7 ml NaOH

%DDA=[7.0ml]*0.1*161 /(0.136*1000)=82.86%



a)



b)

Figure 3. Viscosities of a) traditional and b) cryogenically synthesized chitosans

a) $\eta = K \cdot M^{\alpha}$	*b)* $\eta = K \cdot M^{\alpha}$
$K=1.4 \cdot 10^{-4}$	$K=1.4 \cdot 10^{-4}$
$M^{\alpha}=0.83$	$M^{\alpha}=0.83$
η =3.4	η =4.1
$3.4=1.4 \cdot 10^{-4} \cdot M^{0.83}$	$4.1=1.4 \cdot 10^{-4} \cdot M^{0.83}$
$M^{0.83}$=4.3853	$M^{0.83}$ = 4.4666
M=192112	M=240000

Since chitosan macromolecules have polyelectrolytic properties in the solution, sodium acetate salts were added to the polymer solution to prevent the polyelectrolytic effect [30]. At least 5 measurements were made during each dilution by taking the point of 5-fold dilution of the solutions. The molecular mass of chitosan was determined using the viscometric method. For viscosity determination, 0.2 g of chitosan was dissolved in 0.33 M acetate buffer.

1% solutions of Apis mellifera samples synthesized by traditional (a) and cryogenic (b) methods were prepared and their viscosities were determined using an Ubbelode viscometer and MM was calculated based on viscosity (Fig. 3 a,b).

MM of cryogenically synthesized chitosan was 192 KDa, and conventionally synthesized chitosan was 240 KDa.

Conclusion

Chitosan was synthesized from Apis Mellifera dead bees by cryogenic and conventional methods. The process of deacetylation of chitosan synthesized by the traditional method is carried out for 300 minutes at 100 ± 1 °C, and chitosan synthesized by the cryogenic method is carried out for 120 minutes. In both methods, effective deacetylation occurs, but in the cryogenic synthesis, a large amount of energy is saved and the obtained chitosan has a higher molecular mass and an increase in DDA from 83% to 87%. This is because cryogenic synthesis has better economic results than the traditional method

References

1. Vafa Amirkia, Michael Heinrich, Natural products and drug discovery: a survey of stakeholders in industry and academia// | https://doi.org/10.3389/fphar.2015.00237, 26 October 2015

2. Ikhtiyarova G.A., Kurbonova F.N. Production of carboxymethyl chitosan based on bee podmore and determination of the degree of deacetylation by conductometric titration. NamSU Scientific Bulletin. 2021. No. 11, – P. 94-98 (in Russian).

3. Tsigos, I., Martinou A., Kafetzopoulos D., Bouriotis, V., Chitin deacetylases: new, versatile tools in biotechnology.Trends in Biotechnology 18, 2000. – P. 305-312.

4. Ikhtiyarova G.A., Mengliev A.S., // Determination of the physicochemical properties of chitosan obtained from *Apis mellifera* // Composite Materials Scientific, Technical, and Practical Journal, No. 2/2023, – P. 3-4 (in Russian).

5. Yadav M., Goswami P., Paritosh K., Kumar M., Pareek N., Vivekanand V., Seafood waste: A source for preparation of commercially employable chitin/chitosan materials, Bioresources Bioprocess. 6 (1) (2019) 8.

6. Philibert T., Lee B.H., Fabien N., Current status and new perspectives on chitin and chitosan as functional biopolymers, Appl. Biochem. Biotechnol. 181(4) (2017) – P. 1314 – 1337.

7. Barik M., Bhagya Raj G.V.S., Kumar Dash K., Shams R. A thorough evaluation of chitosan-based packaging film and coating for food product shelf-life extension. Journal of Agriculture and Food Research 16 (2024) 101164. doi.org/10.1016/j.jafr.2024.101164

8. Ikhtiyarova G. A., Turabdjanov S.M., et all Physicochemical properties of chitin and chitosan from died honey bees Apis Mellifera of Uzbekistan. Journal of Critical Reviews. Vol 7., Issue 4, -2020. – P. 120-124.

9. Novikov V.Yu., Konovalova I.N., Dolgopyatova N.V. Chemical Fundamentals of the Technology for Obtaining Chitin and Its Derivatives from the Shell of Crustaceans.* St. Petersburg: GIORD. 2012. – P. 208 (translated from Russian).

10. Rahmadhani D., Zulkifli., Dwi Yuliani K., Frida E.// Hydrophobic and antibacterial properties of textiles using nanocomposite chitosan and SiO_2 from rice husk ash as-coating. South African J. of Chemical Engineering V. 48 2024 P. 366-374 doi.org/10.1016/j.sajce.2024.03.010

11. Phoenix M., Gorrasi S. (2021) Advances in Chitin and Chitosan Science. Molecules 26: 1805.https://doi.org/10.3390/molecules26061805

12. Ding B., Gao H., Song J., Li Y., Zhang L., Cao X., Xu M., Cai J. Tough and Cell-Compatible Chitosan Physical Hydrogels for Mouse Bone Mesenchymal Stem Cells in Vitro. ACS Appl. Mater. Interfaces 2016, 8, 19739–19746, doi:10.1021/acsami.6b05302

13. Pillai C.K.S., Paul W., Sharma C.P. Chitin and chitosan polymers: Chemistry, solubility and fiber formation. Prog. Polym. Sci. 2009, 04.001. 34, – P. 641–678, doi:10.1016/j.progpolymsci.

14. Ikhtiyarova G. A., Kurbonova F.N., Khojiev D.Y.//“Creation of a new model of burns in rats with the determination of their degree and use of Apis Mellifera carboxymethyl chitosan”//Austrian Journal of Technical and Natural Sciences № 5–6 2022 May– June, P.13-17.

15. Kim Tae-Hee, Hu-Lin Jiang, Jere D., In-Kyu Park, Myung-Haing Cho, Jae-Woon Nah, Yun-Jaie Choi, Akaike T., Chong-Su Cho. (2007) Chemical modification of chitosan as a gene carrier in vitro and in vivo. Prog. Polym. Sci. 32 – P. 726–753.

16.Bernkop-Schnurch A., Dunnhaupt S. Chitosan-based drug delivery systems. Eur. J. Pharm. Biopharm. 2012. 81, – P. 463–469, doi:10.1016/j.ejpb.2012.04.007.

17. Liu Y., Yuan Y., Duan S., Li C., Hu B., Liu A., Wu D., Cui H., Lin L., He J., Wu W. Preparation and characterization of chitosan films with three kinds of molecular weight for food packaging. Int. J. Biol. Macromol. 2020. V. 155. – P. 249-259. DOI: 10.1016/j.ijbiomac.2020.03.217.

18. Zhong Y., Zhuang C., Gu W., Zhao Y. Effect of molecular weight on the properties of chitosan films prepared using electrostatic spraying technique. Carbohydr. Polym. 2019. V. 212. – P. 197-205.

19. Rezaei F. S., Sharifianjazi F., Esmaeilkhanian A., Salehi E. Chitosan films and scaffolds for regenerative medicine applications: A review. Carbohydr. Polym. 2021. V. 273. – P. 118631.

20. Lebedeva O.A., Sedelkin V.M., Potehina L.N. Technology for producing and characterizing chitosan nanofiltration membranes. *Izvestiya of Higher Educational Institutions. Chemistry and Chemical Technology*. 2022. Vol. 65. No. 1. – P. 58-65. (in Russian)

21. Chang S.-H., Wu C.-H., Tsai G.-J. Effects of chitosan molecular weight on its antioxidant and antimutagenic properties. Carbohydr. Polym. 2018. V. 181. – P. 1026-1032. DOI: 10.1016/j.carbpol.2017.11.047.

22. Minagawa T., Okamura Y., Shigemasa Y., Minami S., Okamoto Y. Effects of molecular weight and deacetylation degree of chitin/chitosan on wound healing. Carbohydr. Polym. 2007. V. 67. N 4. – P. 640-644. DOI: 10.1016/j.carbpol.2006.07.007.

23. Milusheva R.Yu., Rashidova S.Sh. Chitin, Chitosan from *Bombyx mori* and Nanosystems Based on Them // Fan. − 2016. –P. 54-55. (in Russian)

24. Ikhtiyarova G.A., Hazratova D.A., Umarov B.N., Seytnazarova O.M. Extraction of chitosan from died honey bee Apis mellifera. International scientific and technical journal chemical technology control and management. -Vol. 2020:Iss.2, Article 3. –P. 15-20.

25. Ikhtiyarova G.A., Kuchkorova D.U. Synthesis and Physicochemical Properties of Chitosan from Apis mellifera Bee Subspecies // "Modern Problems of Polymer Science," All-Russian Conference with International Participation, November 13–17, 2023, St. Petersburg, Russia. – P. 416 (in Russian)

26. Ixtiyarova G.A.,. Khaydarova H.A, Khaydarov A.A.//Method of obtaining a chitosan aminopolysaccharide from beat Apis Mellifera. //Chemical Journal of Kazakhstan/2019

27. Here is the translation of the article title and details into English:

28. Ikhtiyarova G.A., Kholto’rayeva N.R, Turabjanov S.M., Aliyeva M.T. Bactericidal Properties of the Chitosan-Silver Complex Extracted from Bees // Journal of Scientific and Technical Progress in Science and Technology. No. 5. – P. 44-48. ISSN 2181-8193. (in Uzbek)

28. Ikhtiyarova G.A., Mamatova Sh.B., Temirov F.F., Kholturaeva N.R. // Synthesis of a Chitosan-Silver Complex from Bee Submora and the Study of Their Physicochemical Properties. Scientific Bulletin of NSU, 2022, Issue 3, pp. 67-73. (in Russian)

29. Ikhtiyarova G.A., Isomitdinova D.S., Saidov J.E.// Chitosan from the death of bees Apis mellifera and complex with silver ions for obtaining antibacterial biofilms// Proceeding X International Conference «Industrial Technologies and Engineering» ICITE – 2023, Volume M. Auezov South Kazakhstan University Shymkent, Kazakhstan November 18, 2023. – P. 110-113.