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# SYNTHESIS, STRUCTURE AND PROPERTIES OF N-CARBOXYMETHYL CHITOSAN

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***Abstract.*** *N-Carboxymethylchitosan (N-CMCS) was obtained by modifying chitosan in the presence of monochloroacetic acid. The optimal reaction conditions were 90°C and 4 hours, with a mass ratio of chloroacetic acid to chitosan of 5:1. The chemical structure and functional groups of the obtained N-CMCs were analyzed using IR spectroscopy. In the region of 1600–1550 cm⁻¹, absorption peaks characteristic of asymmetric stretching vibrations of the carboxylate group (–COO⁻) were observed. Also, the hydrodynamic properties of N-CMCS were studied using the dynamic light scattering (DLS) method, and the hydrodynamic size and distribution parameters of the particles were determined.*

***Keywords:*** *Crab shell chitosan, monochloroacetic acid, carboxymethylchitosan, hydrodynamic diameter*

## N-KARBOKSIMETILXITOZANNING SINTEZI, TUZILISHI VA XOSSALARI

***Annotatsiya.*** *N-Karboksimetilxitozan (N-KMXZ) xitozanni monoxlorsirka kislotasi ishtirokida modifikatsiyalash orqali olindi. Reaksiyaning optimal sharoitlari 90°C va 4 soat ga teng bo'ib, xlorosirka kislotasining xitozanga massa nisbati 5:1 ga teng. Olingan N-KMXZning kimyoviy tuzilishi va funksional guruhlari IQ-spektroskopiya usuli yordamida tahlil qilindi. 1600–1550 sm⁻¹ sohada mos ravishda karboksilat guruhining (–COO⁻) asimmetrik valent tebranishlariga xos yutilish cho'qqilari kuzatildi. Shuningdek, N-KMXZning gidrodinamik xossalari, dinamik yorug'lik sochilishi (DLS) usuli yordamida o'rganilib, zarrachalarning gidrodinamik o'lchamlari va taqsimlanish ko'rsatkichlari aniqlandi.*

***Kalit so'zlar:*** *Crab shell* xitozani, monoxlorsirka kislota, karboksimetilxitozan, gidrodinamik diametr

## СИНТЕЗ, СТРУКТУРА И СВОЙСТВА N-КАРБОКСИМЕТИЛХИТОЗАНА

***Аннотация.*** *N-карбоксиметилхитозан (N-КМХЗ) был получен путем модификации хитозана в присутствии монохлоруксусной кислоты. Оптимальными условиями реакции являлись температура 90 °C и продолжительность 4 ч, при этом массовое соотношение монохлоруксусной кислоты и хитозана составляло 5:1. Химическая структура и функциональные группы синтезированного N-КМХЗ были исследованы методом ИК-спектроскопии. В области 1600–1550 см⁻¹ наблюдались полосы поглощения, характерные для асимметричных валентных колебаний карбоксилатной группы (–COO⁻). Кроме того, гидродинамические свойства N-КМХЗ были изучены методом динамического светорассеяния (DLS), что позволило определить гидродинамический размер частиц и параметры их распределения по размерам.*

***Ключевые слова****: хитозан из панциря краба, монохлоруксусная кислота, карбоксиметилхитозан, гидродинамический диаметр.*

**Introduction.** Chitosan (CS) is a natural polysaccharide obtained from the shells of crustaceans and other marine crustaceans, and is formed by the alkaline deacetylation of the biopolymer chitin. It is characterized as a copolymer consisting of D-glucosamine and N-acetylglucosamine units [1]. The insolubility of CS in water and neutral media significantly limits its scope of application. In order to overcome this drawback, newderivatives of CS are being obtained through various chemical modifications, including carboxymethylation reactions. [2].

Carboxymethyl chitosan (CMCS) is obtained by introducing a carboxymethyl ($–CH_2COOH$) group into the – OH and – $NH_2$ groups of chitosan under the action of monochloroacetic acid (MCA) or sodium monochloroacetate. Depending on the molecular structure, CMCS can be in the form of O-carboxymethyl

chitosan, N-carboxymethyl chitosan, or N,O-carboxymethyl chitosan [3]. Depending on the degree of substitution, solubility, viscosity, and biological activity vary significantly [4].

In the synthesis of CMCS, the reaction temperature, time, pH, and reagent ratio determine the degree of conversion. A conversion degree of more than 50% further improves the antibacterial, antioxidant, and biological properties of CMCS [5]. In addition to the realkylation method, CMCS can also be obtained via the Michael coupling reaction, which provides stereochemical control of the product. [6].

The hydrodynamic properties of CMCS are important in characterizing the behavior of polymer chains in water and determining their practical applications [7], and the hydrodynamic volume affects the ability to form cationic solutions, which provide water solubility and colloidal stability. [8].

The hydrodynamic diameter of CMCS particles measured by DLS was found to be in the range of 300–380 nm, and the zeta potential was found to be around –13 mV [9]. These parameters are crucial in the design of CMCS -based drug delivery systems, especially in the formation of nanoparticles and micelles. The pH-sensitive property of the polymer depends on the degree of ionization of its carboxyl (–COOH) groups, and high solution viscosity is observed in neutral and alkaline media.

IR spectroscopy is one of the main methods for analyzing the structure of CMCS, allowing to determine the degree of chemical modification. In the IR spectrum of the initial CS, a broad absorption spectrum corresponding to the $–NH_2$ group was observed at wavelengths of 3445 and 3422 $cm^{-1}$. In the CMCS spectrum, a strong absorption spectrum corresponding to the symmetric stretching vibration of the $–COO^–$ group appeared at 1412 $cm^{-1}$. In addition, a signal characteristic of the $–CH_2–COOH$ group was also detected at 1480 $cm^{-1}$ [1, 3]. These parameters confirmed the successful completion of the carboxymethylation reaction. From a biomedical point of view, CMCS has a number of important biological properties [11]. In the field of wound healing, CMCS-based hydrogels are being studied as moisture-retaining, bioadhesive, and self-healing materials [12].

CMCS is an active research material in polymer chemistry and biomedicine due to its high-water solubility, large hydrodynamic volume, pH-sensitive properties, and well-characterized IR spectrum. A comprehensive study of their synthesis, hydrodynamic and spectroscopic properties provide a scientific basis for the creation of new highly effective biomaterials and drug systems.

**Research materials and methods.** In carrying out this work, chemical reagents such as "chemically pure" NaOH, $CH_3COOH$, and $CI-CH_2COOH$ from Sigma Aldrich were used.

*IR-spectroscopic analyses.* IR-spectroscopic studies of CS and CMCS were carried out using the IR Tracer-100 spectrometer BRUKER (Germany). IRTracer-100 is characterized by a high signal-to-noise ratio of 60,000:1, a bandwidth of 0.25 $cm^{-1}$, and the ability to operate in a fast scanning mode, allowing recording up to 20 spectra per second.

*DLS measurements.* Particle dispersion, distribution and particle size of CS and CMCS samples in aqueous media were determined by studying dynamic light scattering using a *Photocor* analyzer. This method is based on measuring the average diffusion rate of dispersed particles by recording fluctuations in the intensity of scattered light. This method allows you to calculate the particle size by directly measuring the diffusion coefficient of dispersed particles in solution. The *Photocor* analyzer used allows you to study the processes of formation and decomposition of particles, aggregates, complexes in the temperature range of 278-363 K.

**Results and their discussion.** ***Synthesis of N-carboxymethylchitosan***

3.75 g of monochloroacetic acid was placed in a three-necked flask and dissolved in 15 ml of distilled water. The pH of the resulting solution was adjusted to 7.0 by titration with 10% NaOH. Then, 0.75 g of chitosan was added to the solution. The reaction mixture was heated to 90 °C under vigorous stirring and kept at this temperature for 4 hours. To neutralize the hydrochloric acid (HCl) formed during the reaction and to maintain the pH of the medium at 7.0, a 20% sodium carbonate was added dropwise every 30 minutes. After the reaction was completed, the mixture was filtered to remove unreacted chitosan. The resulting filtrate was diluted with 95% ethanol in a mass ratio of 1:3, and the resulting precipitate was isolated by filtration. The precipitate was washed twice with absolute ethanol and dried under vacuum to obtain N-CMCS.

***IR Spectroscopic Analysis of Chitosan Macromolecule from Crab Shells***

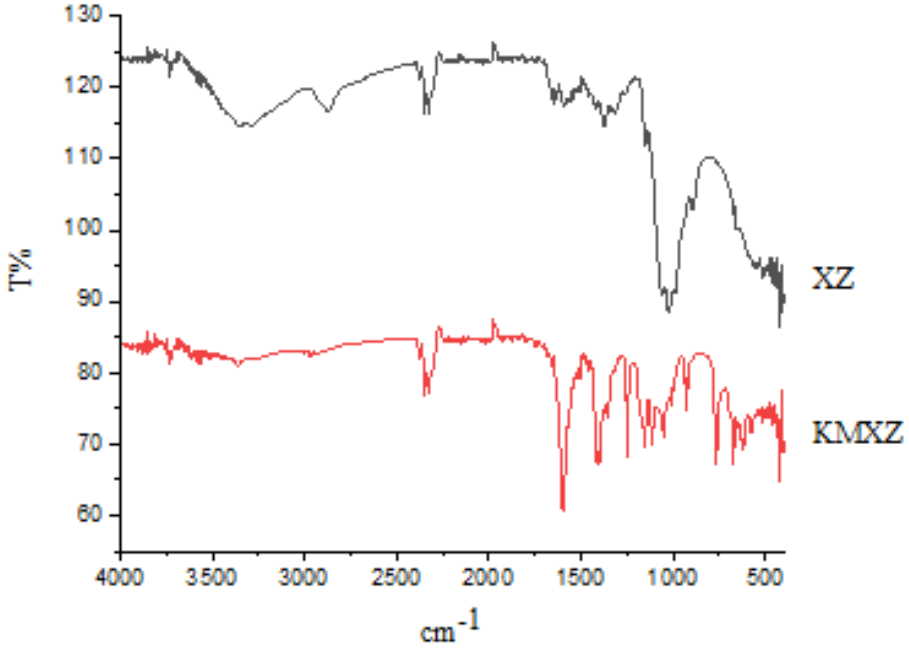



**Figure 1. IR spectra of chitosan and carboxymethylchitosan**

As a result of the analysis of the IR spectra of chitosan and N-carboxymethylchitosan, broad absorption spectra corresponding to the stretching vibrations of hydroxyl (-OH) and amine (-NH) groups were observed in the region of 3200–3500 $cm^{-1}$. As shown in Fig.1, the IR spectrum of chitosan exhibits absorption spectra depending on the saccharide structure in the region of 1155, 1078, 1029 $cm^{-1}$. The peak at 1598 $cm^{-1}$ belongs to $NH_2$.

In the spectrum of N-CMCS, new absorption spectra appeared, confirming the successful completion of the carboxymethylation process. In particular, absorption peaks characteristic of asymmetric and symmetric stretching vibrations of the carboxylate group ($–COO^-$) were observed in the regions of 1600–1550 $cm^{-1}$ and 1420–1400 $cm^{-1}$, respectively. Also, intense absorptions in the range of 1150–1020 $cm^{-1}$ are related to vibrations of the C–O–C and C–O bonds in the polysaccharide chain. The absorption spectra in the region of 900–500 $cm^{-1}$ are explained by deformation vibrations of the carbohydrate ring skeleton.

The appearance of absorption spectra characteristic of the carboxylate group in the spectra confirms the successful modification of chitosan to N-CMCS.

***DLS analysis results***

Particle size in solutions of *Crab Shells* chitosan was examined using a *DLS* analyzer

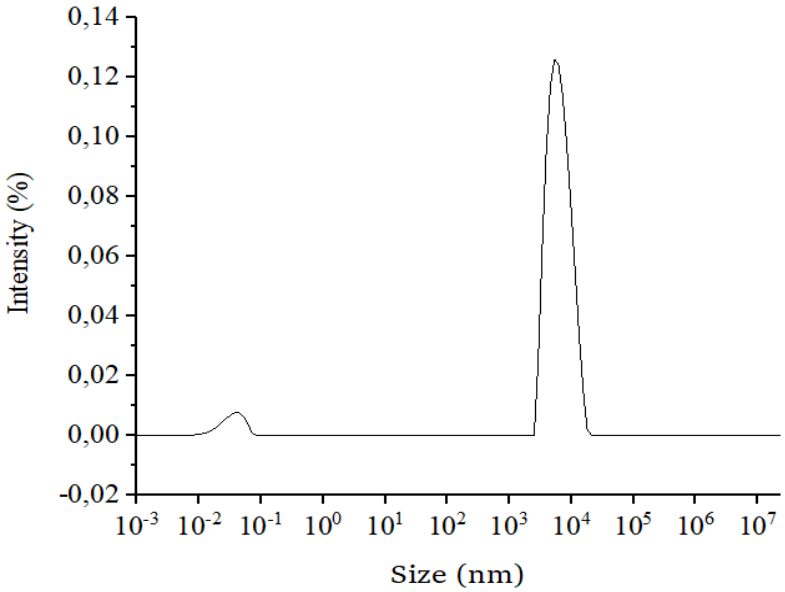



**Figure 2. Dispersion of chitosan particles in solution**

The hydrodynamic size of the obtained N-CMCS particles was characterized using dynamic light scattering DLS. Analysis of the particle size distribution histograms showed that the hydrodynamic diameter of N-CMCS particles in solution varied within a certain range. The particle size distribution showed the presence of particles with the following diameters: 0.04 nm (0.05%) and 7.280 μm (99.5%) Fig.2. It is shown that N-CMCS exhibits a bimodal size distribution characterized by a small fraction of nanoparticles and a large fraction of micrometer-sized particles. This disproportion reflects their aggregation behavior and colloidal stability in solution.

**Conclusion.** Thus, N-CMCS was obtained based on chitosan. The optimal conditions for the synthesis process were found to be a temperature of 90°C, a reaction time of 4 hours and a mass ratio of chloroacetic acid to chitosan of 5:1. Observation of absorption peaks characteristic of asymmetric valence vibrations of the carboxylate group ($–COO^-$) in the region of 1600–1550 $cm^{-1}$ by means of IR-spectroscopy showed that the N-carboxymethylation reaction was successfully carried out. The results of DLS analysis made it possible to determine the hydrodynamic dimensions and particle distribution of N-CMCS. It shows that the synthesized N-CMCS has good solubility in water and is a promising polymer material for drug delivery systems.

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