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OBTAINING OF CHITIN AND CHITOSAN FROM THE NARROW-CLAWED CRAYFISH (PONTASTACUS LEPTODACTYLUS) AND THEIR CHARACTERIZATION BY IR SPECTROSCOPY

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Abstract: Chitosan is a promising auxiliary substance in pharmaceutical technology due to its wide range of shaping and functional properties. A significant reserve of raw material for obtaining chitosan is represented by stocks of the narrow-clawed lake crayfish. Chitosan obtained from narrow-clawed crayfish was studied in order to evaluate its physicochemical and technological properties for the development of pharmaceutical dosage forms. Comparative analysis of the physicochemical characteristics of chitosan derived from narrow-clawed crayfish, bee podmore, and silkworm pupae demonstrated their similar chemical nature and molecular structure. The molecular weight of the obtained chitosan was determined by the viscometric method, while the degree of acetylation was assessed conductometrically. The structures of chitin and chitosan were analyzed using IR spectroscopy.
Keywords: chitosan, IR spectra, physicochemical properties, viscosity, viscometry, conductometry

Introduction

The ability to modify the properties of polymers and to create immobilized compounds on their basis enables the development of pharmaceutical formulations with controlled drug release. The use of hydrophilic, swelling polymers is particularly relevant in this context. These polymers possess not only structuring capabilities but also a wide range of functional properties and high biocompatibility with biological tissues of macroorganisms. Among such materials are polysaccharides and aminopolysaccharides, including chitin and chitosan [1–3].

Chitosan, a poly-(1→4)-2-amino-2-deoxy-β-D-glucose, is produced by removing the acetyl group at the C_2 position of chitin through treatment under harsh alkaline conditions. The appearance of a free amino group in each repeating unit of the macromolecule gives chitosan typical polyelectrolyte characteristics. One of these is the polyelectrolyte swelling effect—an anomalous increase in the viscosity of dilute polymer solutions as polymer concentration decreases [4–7].

The physicochemical and biological properties of this polymer, together with published clinical application data, make chitosan and its derivatives promising substances for the development of pharmaceutical preparations with various pharmacotherapeutic effects [4, 7]. However, a limited number of studies have focused on the functional and technological parameters of chitosan [8].

It is well known that numerous raw material sources and extraction methods exist for obtaining chitin and chitosan. Depending on the type of initial biopolymer, demine-ralization and deacetylation conditions, as well as subsequent processing stages, the molecular weight, degree of acetylation, and biological properties of the resulting product may vary significantly.

Particular interest is directed toward alternative, eco-friendly sources of chitin that allow the production of highly purified chitosan with predetermined physicochemical characteristics. In this regard, the narrow-clawed crayfish (Pontastacus leptodactylus) represents a promising raw material, offering a higher chitin yield and a lower molecular weight of the obtained chitosan compared to traditional sources such as shrimp or crab shells.

The aim of this study was to obtain chitosan from the narrow-clawed crayfish and determine its physicochemical and technological properties for regulatory documentation purposes.

Materials and Methods

For the synthesis of chitin and chitosan, narrow-clawed lake crayfish collected from freshwater reservoirs and lakes of the Bukhara and Navoi regions of the Republic of Uzbekistan were used. Bidistilled water (18 MΩ × cm–1), sodium hydroxide (NaOH), acetic acid (CH_3COOH), and ethanol (C_2H_5OH) were applied in the process. All intermediate products formed during the chemical reactions and incorporated into the final material were not subjected to additional purification.

Viscometry

Viscometry was used to determine the molecular weight and viscosity of the bioorganic polymers. This method was applied to determine the molecular weight of CS. The suppression of the polyelectrolyte effect of CS was achieved by using a 2% sodium chloride solution. The molecular weight of chitosan samples was calculated using the Mark–Kuhn–Houwink equation.

Conductometric Titration

The degree of substitution of bioorganic polymers, including CS, was determined using conductometric titration. For conductometric measurements, we used an instrument manufactured by Mettler-Toledo AG, Analytical CH-8603 Schwerzenbach, Switzerland [9].

IR Spectroscopy

IR spectroscopic analysis of the synthesized biopolymers was carried out using a Fourier-transform infrared spectrometer “IRTracer-100” (SHIMADZU CORP., Japan, 2017), equipped with a MIRacle-10 attenuated total reflection (ATR) accessory with a diamond/ZnSe prism. The spectral range was 4000–400 cm^{-1}, resolution 4 cm^{-1}, signal-to-noise ratio 60,000:1, and a scanning speed of 20 spectra per second.

Results and Discussion

From 1 kg of crayfish collected in freshwater reservoirs and lakes of the Bukhara region, after cleaning, removing the shell, washing, and drying, 42.53 g of purified shell material was obtained.

Demineralization

Ten grams of crushed shell were mixed with 100 mL of 7% hydrochloric acid (solid-to-liquid ratio 1:10) and kept at room temperature (27–28 °C) for 24 hours. The demineralization process was repeated twice. After demineralization, 2.97 g of demineralized raw material remained.

Deproteination

The demineralized shell was treated with 7–10% sodium hydroxide solution (1:10) at 80 °C by placing the material on an asbestos mesh and boiling it for 1.5 hours. This procedure was also repeated twice.

After both stages, 2.428 g of chitin was obtained.

Chitosan Synthesis

The obtained chitin was treated with 50% sodium hydroxide solution (1:10) and boiled for 3–4 hours in a sand bath equipped with an air condenser.
The chitosan was thoroughly washed with distilled water and absolute ethanol until a neutral pH was reached, and then dried at room temperature.
The resulting chitosan was a plate-like, light yellow to brownish, odorless aminopolysaccharide. The mass of the dry product was 1.995 g. The yield of chitosan from crayfish shells was 20%.
Identification of Gammarus chitosan was performed using elemental analysis and IR spectroscopy. Elemental composition (Table 1) showed that chitosan extracted from narrow-clawed lake crayfish has a carbon, hydrogen, and nitrogen ratio similar to that of chitosan derived from bee podmore and close to the theoretical ratio calculated from the chemical formula of chitosan.

Table 1. Selected Physicochemical Parameters of Chitosan Obtained from Various Raw Materials

Sample	Moisture, %	Ash, %	Total nitrogen, %	Solubility in acidic medium, %	Molecular weight, kDa
Chitosan obtained from narrow-clawed crayfish	10.8	0.53	7.01	15	103
Chitosan obtained from bee podmore	10.3	0.58	8.31	10	162
Chitosan obtained from silkworm pupae	9.6	0.62	7.37	9.5	282

The molecular weight of chitosan obtained from narrow-clawed crayfish, determined viscometrically, was 103 kDa.
The degree of acetylation calculated from conductometric titration data was 73%.
For comparative characterization of polymer structure, IR spectra were recorded (Fig. 2).

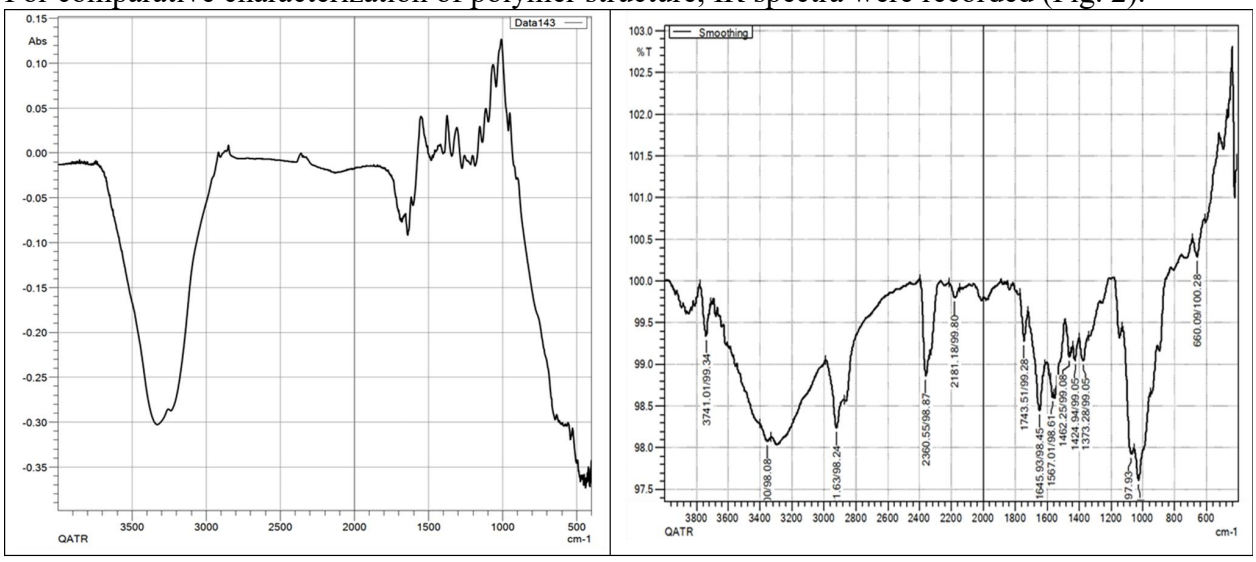

Figure 2. IR spectra of chitosan obtained from bee podmore (A) and narrow-clawed crayfish (B)

In the IR spectra of chitosan, characteristic absorption bands were observed in the regions of 3500–3300 cm^{-1} and 1390–1000 cm^{-1}, indicating the presence of NH_2 groups. Absorption in the

region 3500–3300 cm^{-1} corresponds to N–H stretching vibrations, while the bands in the region 1360–1000 cm^{-1} are typical for amines and result from C–N skeletal vibrations.

In the sample of chitosan derived from narrow-clawed crayfish, absorption maxima were recorded at 1433 cm^{-1}, corresponding to CH- and CH_2-group deformation vibrations, and at 1373 cm^{-1}, corresponding to bending vibrations of O–H bonds. In the chitosan obtained from bee podmore, a broad medium-intensity band was observed in the region 1320–1387 cm^{-1}, corresponding to O–H bond vibrations [5,10,11].

Analysis of the IR spectra confirmed the structural similarity of both chitosan samples. The relatively broad distribution of characteristic bands may be associated with molecular and structural heterogeneity of the studied materials.

Conclusion

The study investigated the specific features of the reactions involved in the production of chitin and chitosan from the narrow-clawed freshwater crayfish (Pontastacus leptodactylus) and identified the kinetic parameters as well as the optimal conditions for chitosan production through the deacetylation reaction. The formation of chitosan was analyzed using physicochemical methods, and the rheological properties of the obtained chitosan were examined.

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