Investigation of some physicochemical properties of chitin from crab shells

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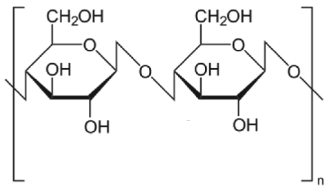
Being considered to be materials of great futuristic potential with immense possibilities for structural modifications to impart desired properties and functions, research and development work on chitin has reached a status of intense activities in many parts of the world. The positive attributes of excellent biocompatibility and biodegradability with ecological safety and low toxicity with versatile biological activities have provided ample opportunities for further development. Because of this reason we have investigated some physicochemical properties of chitin by FTIR spectroscopy, X-ray, termogravimetric analysis.

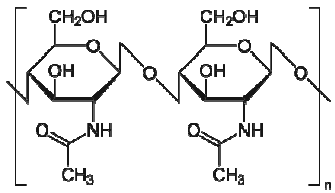
Key wards: *Chitin , FTIR spectroscopy, X-ray, Termogravimetric analysis*

INTRODUCTION

Chitin is one of the most abundant and easily obtained structural biopolymer. It is a linear polysaccharide consisting of 2-acetamido-2-deoxy-β-D-glucopyranose units through a β(1-4)linkage. In spite of the presence of nitrogen, it may be regarded as cellulose with hydroxyl at position C-2 replaced by an acetamido group (Figure 1).



Cellulose $(C_6H_{10}O_5)_n$,



Chitin $(C_8H_{13}O_5N)_n$

Fig. 1. Structures of cellulose and chitin.

Like cellulose, it functions as structural polysaccharides and have three different polymorphic forms (α, β and γ). Its natural production is inexhaustible; cuticles of arthropods, cell walls of most fungi, most by themselves, count more than 10^6 species from the 1.2 x10^6 of total species compiled for animal kingdom, constitute permanent and large biomass source. Chitin is a white, hard, inelastic, nitrogenous polysaccharide and the major source of surface pollution in coastal areas. In its native state chitin is crystalline and possesses different crystalline forms. Chitin is usually isolated from the exoskeletons of crustaceans and more particularly from shrimps and crabs where α-chitin is produced. The α-form has a two-chain unit cell with a $P2_12_12_1$ space group and consequently an antiparallel arrangement of the adjacent chains [10]. β-Chitin is rather rate. In its crystals, the polymer chains are arranged forming a monoclinic $P2_1$ space group with the chitin chain axis as the unique monoclinic axis. It also shows higher solubility, higher reactivity and higher affinity towards solvents and swelling than α-chitin due to much weaker intermolecular hydrogen bonding ascribable to the parallel arrangement of the main chains [2,11].

Potential and usual applications of chitin and its derivatives are estimated to be more than 200, because he possess unique structure, multidimensional properties, highly sophisticated functions [14]. This wide range of applications include cosmetics, agriculture, food, biomedical, and textile, as chelating agents and refinement industrial effluents [3,8,13,15,16].

The aim of the present work is investigation of some physicochemical properties of chitin from crab shell by FTIR spectroscopy, X-ray and termogravimetric analys.

MATERIALS AND METHODS

1.Material

The standard samples of chitin was purchased from Sigma-Aldrich-USA. Before using, chitin was vigorously grounded in agate mortar and dried in air at 60^{o}C for 4 hours.

2. *Fourier transform infrared spectroscopy* (*FTIR*)

IR spectra of chitins obtained from different sources were recorded with a Tensor 27 Fourier transform infrared spectrometer FTIR (Germany). The spectral region between 4000 and 400 cm^{-1} was scanned. Specimens prepared as KBr pellets were used. Dried, powdery chitin was mixed thoroughly with KBr and then pressed in vacuo to homogeneous disc with a thickness of 0.5 mm. The chitin concentration in the samples was 2%, calculated with respect to KBr.

3.Thermal analysis

Differential scanning calorimetry was conducted with a STA 449F1 Jupiter (Germany). A proportion of 6–7 mg of sample dispersions was put into a platinum crucible. The samples were heated from 30 to 800 °C. The experiments were performed in dry air atmosphere, at heating rates of 3, 6, 9, 12, 15 and 18^{o}C min^{-1}.

4. X-ray diffraction

The wide-angle X-ray analysis was applied to detect the crystallinity of chitins prepared and their patterns were recorded using a URD–6 „SIEMENS", (Germany). Data were collected at a scan rate of 1°/min with the scan angle from 5° to 35°. On the base of X-ray analysis by means of strictly measured and analytic observed X-ray absorption bands of the samples researched, in the range $3° \leq 2\theta \leq 60°$ the integral intensity of diffraction reflexes are estimated. The parameters of elementary cells are defined by ITO13-program.

RESULTS AND DISCUSSION

1. FTIR spectrum analys

Various absorption bands within the 4000-400 cm^{-1} range were recorded in the FTIR spectra of chitin, prepared from crab shells [1,5,6,9,12,17,19,20]. The band at 3448 cm^{-1} could be assigned to ν(N-H), ν(O-H) and ν(NH_2) which present in chitin in different amounts among which NH_2 groups being the least. The band at 3267 cm^{-1} is associated with ν(N-H) in secondary amides only with *trans*-configuration and usually is due to the formation of linear associates [20]. *trans*-Configuration of NH-CO group in chitins was confirmed additionally by the lower intensity band at 3110 cm^{-1}. The presence of methyl group in methylene group in CH_2OH and methyne group in pyranose ring was proved by the corresponding stretching vibrations of these groups in the range 2961-2892 cm^{-1} (Figure 2). Studies indicate that chitin, in the crystalline state, shows only one intense peak at 1626 cm^{-1}. However, the spectra of the samples indicated the presence of two bands, one at 1626 cm^{-1} and another at 1657 cm^{-1}, probably, indicating an amorphous state. The band at 1626 cm^{-1} could be attributed to the stretching of C–N vibration, linked to OH group by H bonding. These bands can be clearly observed in all samples. When these two peaks appeared with certain intensity, we observed two bands at 1626 and 1656 cm^{-1}. The wide peak at 3500 and 1650 cm^{-1} indicated that the hydrogen interactions are less accentuated, or the presence of free hydroxyl groups [4]. The sharp band at 1379 cm^{-1} corresponds to a symmetrical deformation of the CH_3 group, and at 1559 cm^{-1} corresponds to the stretching or N–H deformation of amine II [4,18]. The absorption bands within the 1420-700 cm^{-1}

region confirmed the presence of CH_3, CH_2 and CH groups as well as the primary and secondary OH groups, attached to the pyranose ring, and the oxygen atoms in ether groups.

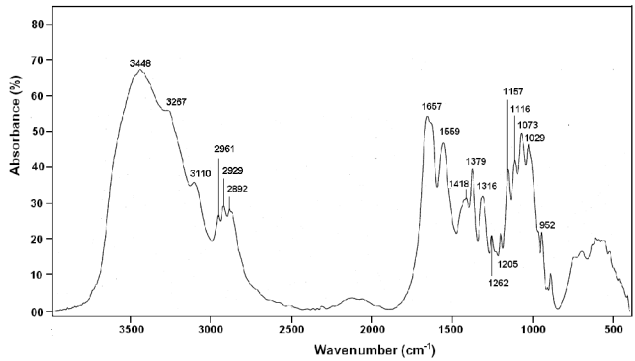


Figure 2. . FTIR spectra of chitins prepared from Crab shell

2. Thermogravimetric analys

TG profile of pyrolysis for chitin at various heating rates in the temperature range of 500-700 K. On Fig. 3 are presented the TG, DTG and DTA curves, obtained at the thermal degradation of chitin at a heating rate of 6°C min^{-1}.

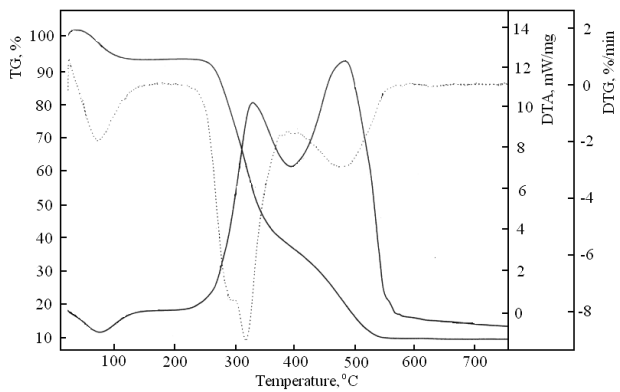


Figure 3. . TG, DTG and DTA curves, obtained at the thermal degradation of chitin at a heating rate of 6 °C min^{-1}.

In the thermogram of chitin two decomposition steps could be observed, the first occurs in the range of 50–110°C, and is attributed to water evaporation. The second occurs in the range of 300–400°C and could be attributed to the degradation of the saccharide structure of the molecule, including the dehydration of saccharide rings and the polymerization and decomposition of the acetylated and deacetylated units of chitin. At a temperature of 380°C is observed significant change in the course of the TG-curve. It is may be due to the change of the mechanism of the degradation process. In the DTG and DTA-curves are observed two peaks - at 315 and 453°C respectively. These two stages are strongly exothermic. The first stage ended at 380°C and is connected with 56.5% mass loss, but the second one - with 27% mass loss. The last corresponds to the residual cross-linked degradation of chitosan.

3. X-ray diffraction analysis

X-ray diffraction (XRD) analysis was applied to detect the crystallinity of the isolated chitin and the obtained chitosan. The structure of α-chitin has been determined by using X-ray diffraction analys, based on the intensity data from deproteinized crab shells. Least-squares refinement shows that adjacent chains have alternating sense (i.e. are antiparallel). In addition, there is a statistical distribution of side-chain orientations, such that all the hydroxyl groups form hydrogen bonds. The unit cell is orthorhombic. The chains form hydrogenbonded sheets linked by C=O…H–N bonds are approximately parallel to the α-axis, and each chain has an O-3'–H…O.5 intra-molecular hydrogen bond, similar to that in cellulose. The results indicate also that a statistical mixture of CH_2OH orientations is present, equivalent to half oxygen on each residue, each forming inter- and intra-molecular hydrogen bonds. As a result, the structure contains two types of amide groups, which differ in their hydrogen bonding, and account for the splitting of the amide I band in the infrared spectrum. The inability of this chitin polymorph to swell on soaking in water is explained by the extensive intermolecular hydrogen bonding. In Fig. 4, the X-ray diffraction patterns of the obtained α-chitin is given.

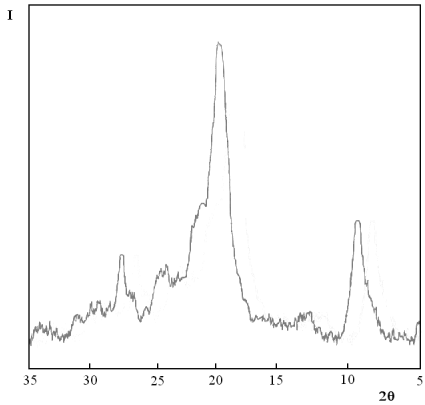


Figure 4. X-ray diagram of chitin

In the DTG and DTA-curves are observed two peaks - at 315 and 453°C respectively. These two stages are strongly exothermic. The first stage ended at 380°C and is connected with 56.5 % mass loss, but the second one - with 27% mass loss. The last corresponds to the residual cross-linked degradation of chitosan. Chitin sample show strong reflections at 2θ around 9 and 2θ of 18–20° and minor reflections at higher 2θ values e.g. at 24.8° and higher. The band at 9° corresponds to a d spacing of 8.92 Å and is due to the incorporation of bound water molecules into the crystal lattice. The reflection at 2h 19.4–20° corresponds to a d spacing of about 4.41 Å [7].

The starting values of crystalline peaks positions were calculated from the unit cell dimensions of α-chitin given by Blackwell [10]. According to [10], the unit cell of α-chitin is orthorhombic with dimensions a=0.474 nm, b=1.886 nm, c=1.032 nm.

3. CONCLUSIONS

Chitin have a wide range of applications. By employing FTIR spestroscopy, all functional groups in chitin macromolecules are elucidated. Our investigations in this work performed and have shown that the basic range of the thermal degradation of chitin is 300-500°C. XRD investigations have proved that apart from the type of the crystalline structure

of chitin, the size and perfection of crystallites are the most important factors influencing is thermal stability.

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Докладът е рецензиран.