A comparative investigation of the structure and properties of nanofibers based on *Bombyx mori* chitosan and *Artemia sp.cysts* chitosan

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Abstract. The *Bombyx mori* chitosan and *Artemia sp.cysts* chitosan were isolated from different sources, and nanofibers based on them were electrospun from the acetic acid solutions. The rheological properties of chitosan solution in 80% acetic acid were investigated. The structure and morphology of the nanofibers were characterized by Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction, and scanning electron microscope (SEM). It was revealed that the structure of chitosan samples changes when producing nanofibers by electrospinning.

1 Introduction

Thanks to their biological activity, biocompatibility, nontoxicity, and other unique properties, native polysaccharides such as chitin and chitosan have the high attention of specialists in various fields [1].

Chitosan is synthesized by deacetylation of chitin, which can be isolated from different sources: crustaceans, silkworm pupae, insects, and others [2-3]. Electrospinning nanofibers from polymer solutions is a new approach to obtaining nanotechnology materials. The advantage of the method is that the resulting nanofibers are characterized by porosity and a highly developed structure, which determines their use. These nanofibers find applications in biomedical applications, such as wound dressings and antibacterial coatings, as well as to provide antimicrobial and antiviral barrier properties, scaffolds for tissue engineering, regulation of water permeability, vapour permeability, and drug delivery [4,5].

Quite a lot of attention from scientists is paid to the process of electrospinning chitosan from crustaceans [6]. The works [7, 8] showed the possibility of obtaining nanofibers from solutions of chitosan and found that the most suitable solvent is acetic acid with a concentration of 80–90%. There are some papers where various additives are used to improve the properties of solutions or to modify a final nonwoven material with good biodegradability and biocompatibility [9, 10]. Despite a sufficient number of publications on the production

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of nanofibers from crustacean's chitosan, the process of electrospinning from other sources of raw materials has not been sufficiently studied.

In this work, nanofibers were obtained from Bombyx mori (Bm Chz) and Artemia sp. cystis chitosans, as well as studied for their structural characteristics.

2 Materials and methods

Two types of chitosan were used for electrospunning nanofibers: *Bombyx mori* chitosan and *Artemia sp. cystis* chitosan, with molecular weights of 184 kDa and 90 kDa, respectively, that were determined by using viscosity method and calculated by the Mark-Kuhn-Hauwink equation [11]. The rheological properties of the electrospunning chitosan solutions was studied by the MCR 92 Rheometer (Anton Paar, Austria) using a system of coaxial cylinders in a shear flow at 25°C.

The NanoNC eS-robots device was used for electroforming nanofibers and the process conditions were following: voltage 25 kV, solution flow rate 30 µl/min, distance between anode and cathode 14 cm.

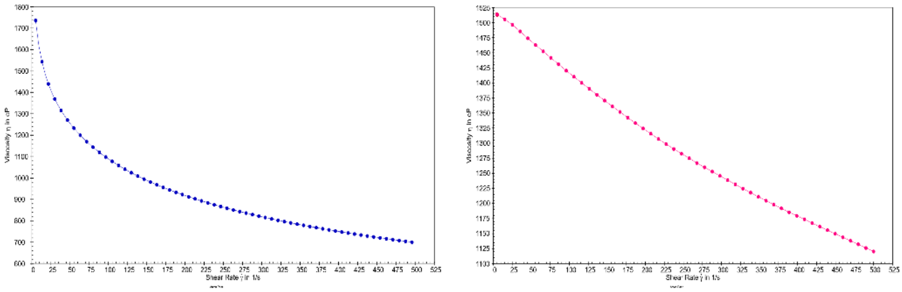
FTIR spectra of nanofibers were recorded using Inventio-S (Bruker, Germany) in 400-4000 cm^{-1} wavenumber range.

The nanofibers morphology was investigated by using Scanning electron microscopy and the studies were performed using SEM equipment Veritas-3100 (Korea). Magnification of the device x10-300000, voltage 200V-300V, maximum scanning area (x÷y÷z) is 120÷120÷65 µm.

XRD Miniflex 600 (Rigaku, Japan) was used to study the amorphous-crystalline structure of nanofibers. The spectrum was recorded in the interval 2θ=2°–70°. The software "SmartLab Studio II" was applied for the treatment of experimental results.

3 Results and discussion

The rheological behaviour of the samples showed a difference, i.e., the viscosity curve of *Bombyx mori* chitosan solution is sloping, whereas the *Artemia sp. cystis* chitosan solution viscosity curve is slightly sloping. It shows the *Bombyx mori* chitosan solution has a non-Newtonian nature, i.e. its viscosity is weakly dependent on the applied speed or shear stress. It can be related to the deformation ordering of chitosan macromolecules in a wide range of the shear field velocity gradient (Figure 1a).



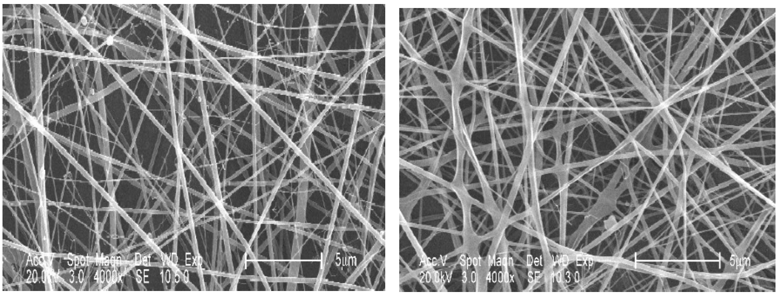
a b

Fig. 1. Dependence of the viscosity (η) on the shear rate (γ) (in the logarithmic coordinate): a) *Bombyx mori* chitosan solution and b) *Artemia sp.cysts* chitosan solution.

It was found the *Bombyx mori* chitosan solution has a thixotropic property and forms hysteresis loops, and their width determines a relative estimate of the degree of structure-forming processes, which has the value A=2039.6 Pa/s.

The *Artemia sp.cysts* chitosan solution has a Newtonian character, i.e. viscosity is practically independent of the magnitude of the applied stress or shear rate. It may be due to the molecular weight of macromolecules which, in this range of the shear rate, no conformational change is observed (Figure 1b). The average viscosity for a chitosan solution of *Artemia sp.cysts* is 1253 mPa s.

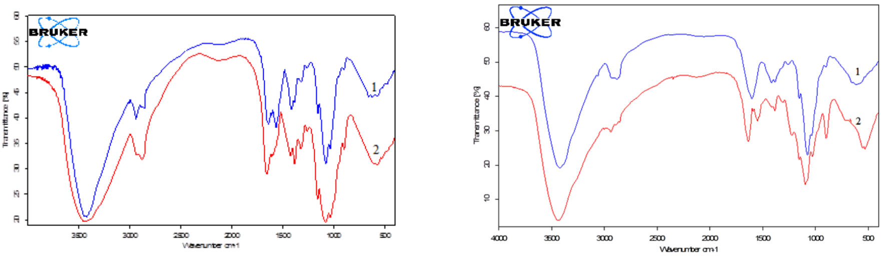
SEM studies showed-that the thickness of the resulting *Bombyx mori* chitosan nanofibers ranges from 100-600 nm, but the nanofibers have an ideal cylindrical shape. In chitosan nanofibers from *Artemia sp.cysts*, along with cylindrical nanofibers, there are sections in the form of nodes and have a narrower thickness distribution, the sizes of which range from 90-450 nm (Figure 1 a, b).



a *b*

Fig. 2. SEM images of nanofibers: *a*) *Bombyx mori*; *b*) *Artemia sp. Cysts.*

The FTIR spectroscopy results showed that the Bombyx mori chitosan structure was changed after forming nanofibers. During the electrospinning of nanofibers from polymer solutions, chitosan macromolecules are oriented under high voltage (Figure 3), which changes the crystallinity and polymorphism of chitosan. It resulted in slight differences in the absorption band intensity at 1604 cm^{-1} and 1592 cm^{-1}. In nanofibers, a narrower absorption band at about 3500 cm^{-1} is observed, which confirms the partial participation of hydroxyl groups in the C_2 and C_6 positions of chitosan in intra- and intermolecular hydrogen bonds. The absorption band above 3000 cm^{-1} also shifts to a higher frequency, demonstrating a higher-order structure for these samples. It was detected that the bands of stretching vibrations of the CH_2 groups at about 1425 cm^{-1} were more intense than those of the initial chitosan.



a *b*

Fig. 3. FTIR spectra of chitosan nanofibers: *a*) *Bombyx mori*; *1-nanofiber, 2- initial b*) *Artemia sp. Cysts; 1- initial, 2-nanofiber.*

FTIR spectroscopy studies of Artemia sp. cysts chitosan nanofibers were also carried out in comparison with the original chitosan. It was observed that there was a narrow absorption band around 3443 cm^{-1} in the spectrum of nanofibers, which likely confirms the partial participation of hydroxyl groups at C2 and C6 in intra- and intermolecular hydrogen bonds. The higher ordering of the nanofiber structure is evidenced by a shift of the absorption band at 3000 cm^{-1} to a higher frequency. Differences are observed in the region of absorption bands of 1636 cm^{-1} and 1590 cm^{-1}; in nanofibers, the intensity of the amide-II band increases and the amide-I band disappears.

X-ray diffraction analysis of a sample of *Artemia sp. cysts* chitosan showed, the crystalline reflections are observed at 2θ = 4.9°, 10.3°, 16.9°, 19.6°, 22.3°, 26.4°, 28.4°, 38.3°, 41.8° and in the considered range of scattering angles different from the original chitosan [12]. In contrast to the original *Artemia sp. cysts* chitosan, it was observed the crystals with triclinic and orthorhombic systems, which are associated with the conditions of electrospinning nanofibers.

Chitosan with orthorhombic system has lattice parameters: a=8.65 Å, b=15.60 Å, c=10.88 Å, α=β=γ=90.00°, and with triclinic system it has lattice parameters a=3.04 Å, b=9.59 Å, c =17.82 Å, α=93.80°, β=90.34°, γ=98.11°, which also differ from the lattice parameters of the *Artemia sp. cysts* chitosan. This may be due to orientation effects during the electrospinning process. Under the influence of a high electrostatic field, the chains of chitosan macromolecules are oriented in one direction, and this leads to the formation of crystals with parallel chains, in which the value of the lattice parameters increases to 15%. In this case, the size of the crystallites increases by an order of magnitude in the [002] direction and decreases in the [011] direction, from which one can judge the orientation of the macromolecules along the axis direction.

In the X-ray diffraction patterns of *Bombyx mori* chitosan nanofibers, crystalline reflections with maxima at 2θ = 12.6°, 17.67°, 20.42°, 22.88°, 29.08°, 38.76° and 42.68° are observed. The most intense maximum is observed at an angle of 2θ=38.76°, which relates to the crystallographic reflection (032) (Figure 4). In the chitosan structure crystals with triclinic and monoclinic syngonies coexist, which are associated with deacetylation conditions. Chitosan with triclinic system has lattice parameters a=2.63 Å, b=9.01 Å, c=16.19 Å, α=75.80°, β=105.00°, γ=98.00°. The crystallinity index of chitosan decreases during the obtaining process from chitin, which is in good agreement with literature data [12,13-15].

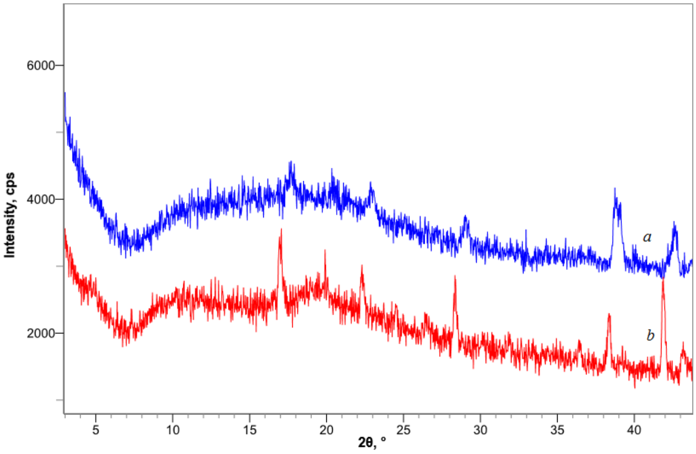


Fig. 4. X-ray diffractions of chitosan nanofibres: a) *Bombyx mori*; b) *cysts of Artemia sp.*

It is observed a change in the intensity and width of the coherent scattering region for *Bombyx mori* chitosan nanofibers, and in the process of identifying the unit cell parameters, it was established that during electrospinning, the system of initial samples changes and has an orthorhombic system with cell parameters a = 10.6 Å, b = 4.62 Å and c = 3.99 Å.

Moreover, in the case of *Bombyx mori* chitosan, the crystallite sizes increase three times in the [002] direction and decrease in the [032] direction, which indicates the orientation of macromolecules along the axis direction.

It was detected the size of crystallites increases during the electroforming process, and this could be explained by more favorable conditions for the improvement of crystallites.

4 Conclusion

Thus, the nanofibers were isolated from *Bombyx mori* chitosan and *Artemia sp.cysts* chitosan, and their structure and morphology were investigated. Resulting *Bombyx mori* chitosan nanofibers ranges from 100-600 nm, but the nanofibers have an ideal cylindrical shape. In chitosan nanofibers from *Artemia sp.cysts*, along with cylindrical nanofibers, there are sections in the form of nodes and have a narrower thickness distribution, the sizes of which range from 90-450 nm.

During the electrospinning of chitosan solutions from various raw materials under the influence of a high electrostatic field, the chains of chitosan macromolecules are oriented in one direction, and this leads to the formation of crystals with parallel chains, in which the value of the lattice parameters increases to 15%. It is shown that, in this case, the size of the crystallites increases by an order of magnitude in the [002] direction, and decreases in the [011] direction from which one can judge the orientation of the macromolecules along the axis direction. Moreover, in the case of Bombyx mori chitosan nanofibers, the crystallite sizes increase three times in the [002] direction and decrease in the [032] direction, which indicates the orientation of macromolecules along the axis direction.

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