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Bionanocomposite Films Based on Chitosan with Bentonite Clay and Polyvinyl Alcohol

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Abstract

Nanocomposite films based on chitosan nanowhisker (CsW) with ionic and nonorganic polymers were prepared and studied on the swelling and mechanical properties. Chitosan nanowhiskers were made from α-chitin by using a top-down approach. Ionic polymer polyvinyl alcohol (PVA) and nonorganic bentonite clay (BC) were employed to prepare chitosan nanowhisker bionanocomposite film taking into account their biocompatibility and nontoxicity. Fourier transform infrared spectroscopy analysis, swelling degree measurements and mechanical tests were employed to analyze the influence of significant PVA and bentonite amounts on the film properties. The mechanical strength was found to increase with the rise of the PVA concentration. This is an advantage for producing wound dressing material. The increase in BC concentration in the film resulted in high film stability in water and different mediums and in higher water absorbance than that of CsW/PVA film. However, the highest swelling degree was for a film of CsW itself but the mechanical strength was lower and insufficient to use it for the wound dressing material. Thus, the incorporation of PVA and BC into chitosan nanowhisker can enhance the swelling capacity and mechanical strength.

1. Introduction

The development and creation of nanotechnologies and nanomaterials is a leading trend in all countries of the world, and the scope of their application is huge – ranging from technology to medicine. The development of nanotechnology in biomedicine is connected with the improvement of technologies and methods of visualization, characteristics and analysis of biomaterials that provides a high degree of resolution of the order less than 10^{-6} m [1].

Over the past two years, the research team has shown scientific interest in widespread biopolymer-chitin and its derivative chitosan nanomaterial, which has some valuable properties, availability and harmlessness, and can be widely used in biomedicine especially considering its sorption, lipotropic, bacteriostatic, immunomodulatory and other biological activity [2, 3].

Analysis of scientific research indicates that the cationic nature of chitosan allows the formation of complexes with anions due to the presence of a large number of amine groups, which contributes to various biomedical applications in the form of scaffolds for tissue engineering, drug carriers and delivery, surgical threads, bone healing, and especially as a dressing material for wound healing due to its antimicrobial activity, tissue regenerating, and wound healing properties [4–6].

Nanoparticle production techniques can be broadly classified into two main categories: the “top-down” and the “bottom-up” approaches. In the “top-down” approach, the size of the particle is reduced from bulk to nanoscale during the production process. As for the “bottom-up” approach, the particle size is assembled from the atomic scale to the nanoscale by various approaches. In the research work, the “top-down” approach was employed for the preparation of nanoparticles (nanocrystals)

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or nanofibers due to the simplicity of producing highly crystalline nanomaterials compared to the "bottom-up" process, which requires the complete dissolution of chitin at the molecular level, which is difficult [7, 8]. The research of Tran et al. shows the preparation and their characteristics of 5 chitin nanomaterials prepared via top-down approaches, one of them is chitosan nanowhisker [9]. The research indicates that the chitosan nanowhisker (CsW) has improved aqueous dispersibility, high antibacterial property and thermal degradation stabilities.

So it can be seen that it is essential to synthesize more affordable wound dressing materials with increased physico-chemical, sorption-desorption and mechanical properties. Using the chitosan nanowhisker with ionic polymer and bentonite clay is a potential favorite in this regard. Such compositions have improved thermal, mechanical and sorption properties at a very low content of the mineral component (3–6%) compared to monopolymers or typical composites. In the study of natural bentonite deposits in East Kazakhstan, the following advantages were identified: high sorbtion properties, availability (deposits located near consumers), low price, resistance to temperature and climatic conditions in transportation, storage and use. This mineral consists of a crystal lattice with three layers. The two outer layers are composed of a silicon-oxygen lattice with silicon atoms in the middle, and the inner layer contains hydroxyl groups with tightly bound oxygen atoms and aluminum atoms. The combination of these three layers forms layered sets that are connected by Na, Ca, Mg, K, and water transition cations. In the process of hydrating sodium bentonite, the forces of the bonds are destroyed, the aggregates break down into particles, and the total volume of clay increases (14–16 times). One of the most important characteristics of clay minerals is their dispersion. Montmorillonite clays are characterized by high dispersion and the dispersion of bentonite in water was determined by the Figurovsky method [10]. According to the sedimentation analysis, the studied clay was found to consist of one mineral and have a high dispersion ($r \approx 0.3 \cdot 10^{-5}$ m). According to the method of swelling equilibrium, the degree of swelling of clay in water was 16 g/g per day [11]. This indicator also showed that the Manyrak's clay belongs to the Na-montmorillonites. The obtained results and literary information indicate that the bentonite clay of the studied area consists of the mineral montmorillonite with high dispersion.

Thus, in this research bentonite clay (BC) from East Kazakhstan, Manyrak filed and PVA was employed for the formation of bionanocomposites, that can improve the sorption ability, surface area and mechanical strength [11–14].

In the given study, the chitosan nanowhisker was synthesized by the "top-down" method from α-chitin biomass and the two biocomposite materials were produced based on CsW/PVA and CsW/BC. The physico-chemical characteristics, swelling degree and mechanical properties to create anti-burn, wound-healing materials were analyzed.

2. Experimental part

2.1. Materials

α-Chitin was purchased firm "Titan Biotech" (India). Poly (vinyl alcohol), [-CH_2CHOH]n, (average MW is 85.000–124.000, 98–99% hydrolyzed) was purchased at the firm "Sigma-Aldrich", (United States). Bentonite clay (theoretical formula $Al\ [OH]_2\{Si_4O_{10}\}\ mH_2O$) was delivered from the East Kazakhstan region, Manyrak deposit. Sodium hydroxide (NaOH) was purchased at the firm "Laborfarma LLP", (China). Aqueous acetic acid was 2 wt.%.

2.2. Preparation of Chitosan nanowhiskers

CsWs were obtained by hydrolysis of chitin nanofiber using 3 M NaOH aqueous solution at 80 °C for 6 h under reflux. The reaction mixture was centrifuged two times, each at 5000 rpm for 20 min at 10 °C. After the centrifugation, the product was rinsed with distilled water, neutralized by 0.5 M NaOH solution, and dialyzed for 1 week. After the purification, the chitosan solution was dried by the freeze-drawn method.

2.3. Preparation of CsW film

The chitosan powder was dissolved in 2 wt.% of aqueous acetic acid for 2 h at 80 °C and was used for the preparation of films with PVA and bentonite clay.

2.4. Preparation of PVA film

PVA was dissolved in distilled water for 1 h at 100 °C and cast in a petri dish with a diameter of 22 cm.

2.5. Preparation of BC film

BC was dispersed into the distilled water for 2 h at room temperature and cast in a petri dish with a diameter of 22 cm.

2.6. Preparation of CsW/PVA films

Polyvinyl alcohol (PVA) solution (10 wt.%) was prepared by dissolving PVA powder in distilled water at 100 °C. The solution was dissolved for 4 h and mixed with chitosan nanowhisker solution for 30 min. After that, the mixture was sonicated for 1 h to remove all trapped bubbles and cast and dry in a plastic petri dish following the casting method. Three films were obtained with various ratios of chitosan and PVA, which are 1:1, 1:2 and 2:1.

2.7. Preparation of CsW/BC films

Bentonite clay solution (BC) (2 wt.%) was prepared by dissolving bentonite powder in distilled water for 2 h. The solution was mixed with chitosan nanowhisker for 30 min and sonicated for 1 h to remove all trapped bubbles. The solutions were cast in a plastic petri dish and dried at room temperature for 1 day. Three films were obtained with various ratios of chitosan and BC, which accounted 1:1, 1:2 and 2:1. Figure 1 shows the principle scheme of preparing CsW/PVA and CsW/BC bionanocomposites.

2.8. Infrared Spectroscopy

For all the synthesized films the IR spectra were taken with a spectrophotometer FSM-1201 ("Lomo"), St. Petersburg with the set of tableting with KBr.

2.9. Swelling degree in water and at pH 3, pH 9

The pure chitosan nanowhisker, PVA and BC as well as bionanocomposite films were cut into small pieces with 20×20×0.1 mm dimensions and weighed (m_0). Then, the samples were immersed in distilled water at room temperature. The swelling analysis was made after each 5, 10, 15, 30, 60, 90 min and 3, 6, 24, 48, 72 h. After each measurement, the swollen film samples were removed from distilled water, measured, weighed and immersed back in the water. The degree of swelling (α) was calculated as follows:

$$\alpha = \frac{m - m_0}{m_0}$$

2.10. Tensile strength

Tensile tests were performed using a texture analysis instrument (Stable Micro System, Great Britain). The CsW, CsW/PVA and CsW/BC films were cut into a rectangle shape with a width of 10 mm and length 50 mm.

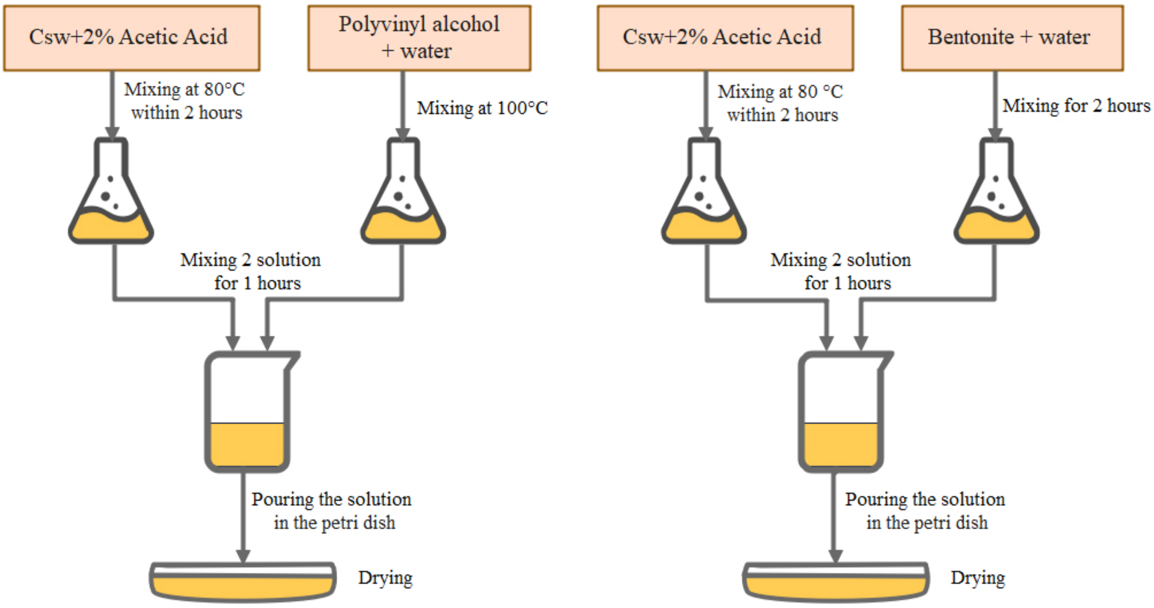
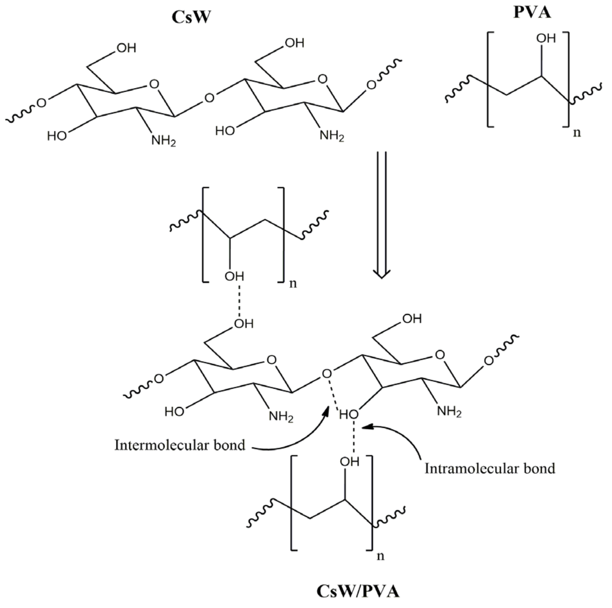


Fig. 1. Principle scheme of preparing biomaterials based on CsW with BC and PVA.

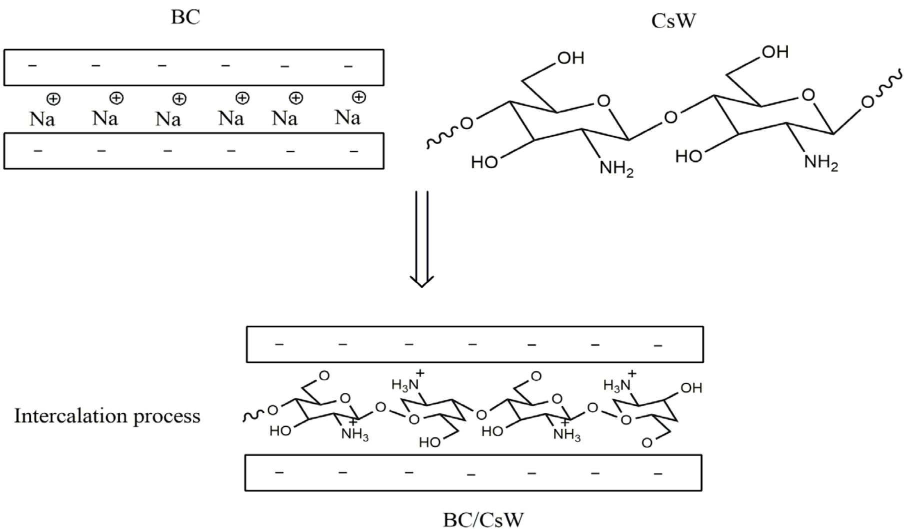
3. Results and discussion

PVA is a non-ionic synthetic polymer, which is soluble in water. PVA has a hydroxyl group in the structure due to which it reacts with other functional groups. Moreover, PVA is a nontoxic and biocompatible. Thus, chitosan and PVA can be crosslinked to each other. The primary hydroxyl group at C6 is linked together with the hydroxyl group of PVA by hydrogen bond that is an intramolecular hydrogen bond. Moreover, the hydroxyl group at C5 and at C4 in the chitosan structure forms a intermolecular hydrogen bond with each other [15]. Scheme 1 shows the mechanism of chitosan interaction with PVA.

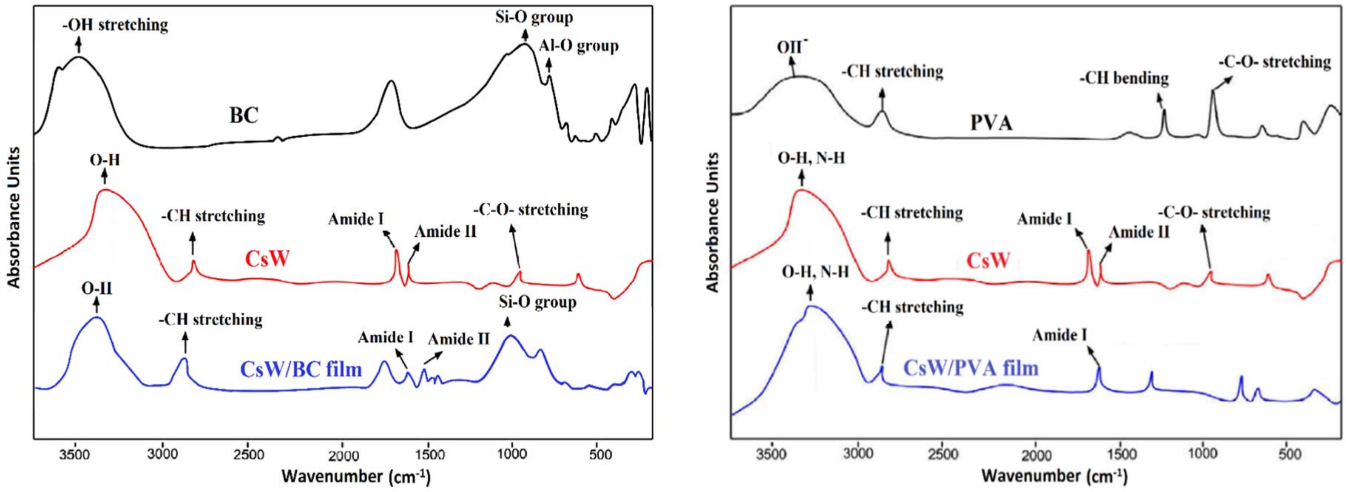


Scheme 1. Proposed mechanism of interaction between chitosan nanowhisker and polyvinyl alcohol.

Scheme 2 indicates an interaction between clay and chitosan. Depending on the degree of the distribution of clay particles in the polymer matrix of the composite, three basic structures are distinguished. There is a micro composite (polymer surrounds agglomerates of clay), an intercalated composite (penetration of the polymer into the interlayer space of the clay, an expansion of the layers to 2–3 nm, partial stratification) and an exfoliated composite (complete exfoliation of clay layers) [16, 17]. At the same time, the formation of a composite with a mixed structure often occurs. The excess of clay and a poor degree of dispersion leads to the presence of mineral agglomerates in the polymer matrix. Moreover, there is a strong impact on both the supramolecular and molecular structure of the polymer and the process of polymer formation. These characteristics of the polymer clay compositions are provided by the non-Coulomb nature of their interaction and the formation of polymer clay composition due to hydrogen bonds which are stabilized by hydrophobic interactions. The studies indicate that bentonite interacts electrostatically with cationic polymers which entails the interaction of cationic polymers between layers. So BC is a good choice for intercalation with chitosan for modification. The amine group of chitosan in an acidic solution turns into cationic form NH_3^+, which is essential for the cation exchange reaction between the bentonite clay and an intercalant positive charged polymer (Scheme 2) [18]. So, the negatively charged bentonite interacts with amine positive charged chitosan by the intercalation process.



Scheme 2. Proposed mechanism of interaction between chitosan nanowhisker and bentonite clay.



(a) (b)

Fig. 2. IR spectra of the composite CsW/PVA (a) and CsW/BC (b) films.

To obtain further evidence on the composite formation, FTIR spectra of raw BC, PVA, CsW and CsW/PVA, CsW/BC composite were recorded in the region of 4000–600 cm^{-1}. The results of IR spectroscopy show the formation of the CsW/PVA and CsW/BC complex.

Figure 2 shows IR spectra of pure PVA, pure chitosan and CsW/PVA with equal ratios. The pure PVA has the main absorption peaks at 3412 cm^{-1} (–OH stretching), 2862 cm^{-1} (–CH stretching), 1419 cm^{-1} (–CH bending) and 1093 cm^{-1} (–C–O– stretching). In the IR spectrum of CsW/PVA film, the characteristic peaks of PVA and chitosan are apparent and the broad band at 3380–3412 cm^{-1} corresponds to the stretching vibrations of hydrogen bonded O–H and N–H shifted to lower wavenumbers. It presumes a crosslinking reaction between hydroxyl groups in PVA and CsW chains [19].

The second scheme demonstrates the spectra of raw bentonite, pure chitosan and CsW/BC with equal ratios. The main absorption bands of BC have appeared at 986 cm^{-1}, which corresponds with the stretching vibration frequency of the Si-O; 1635 cm^{-1}, which is associated with the bending frequency of water molecules absorbed on clay surface; 3400 cm^{-1}, corresponding to the stretching vibration frequency of O-H water molecules were adsorbed onto BC and a band centered at 3621 cm^{-1}, related to the stretching vibrations of structural O-H groups [20]. The comparison of the BC and CsW/BC infrared spectra indicates changes in specific band absorption intensities. The reduction in Si-O band intensity is attributed to the weakening of Si-O bonds due to the formation of hydrogen bonds with CsW [21]. Moreover, new bands related to the absorption of CsW molecules appeared in the spectra of CsW/BC. Bands centered at 2885 and 2946 cm^{-1} correspond to the symmetric and antisymmetric stretching vibration frequency of C-H at CH_3 groups presented in the N-acetyl groups. The peaks of $-NH_2$ groups in pristine CsW at 1550 cm^{-1} are shifted to 1535 cm^{-1} (CsW/BC) which leads to the lower frequency position. The band of the protonated amino group was observed because $-NH_3^+$ groups interact electro-statically with the negatively charged sites of the clay. These results revealed the intercalation of chitosan in the bentonite structure.

Water absorption capacity is quite important for all wound dressing materials because that helps to avoid dehydration of tissue, slows down the growth of microorganism, and also saves wound maceration. So, one of the important properties of films is their swelling ability. The swelling capacity in the free volume for the obtained composites was investigated by the gravimetric method in a stationary mode. The swelling behavior was studied for three days in water and 6 h in an acidic and alkaline environment (Fig. 3). An analysis of the swelling kinetics of CsW/PVA composite films in water shows general patterns: the equilibrium value of the degree of swelling is established within a day, and then it gradually increases ($\alpha \sim 9–15$ g/g); with an increase in ionic polymers, the degree of swelling in water decreases, and acidic and alkaline media significantly affect the kinetics of film swelling; the degree of swelling of composite films is lower than that of chitosan films.

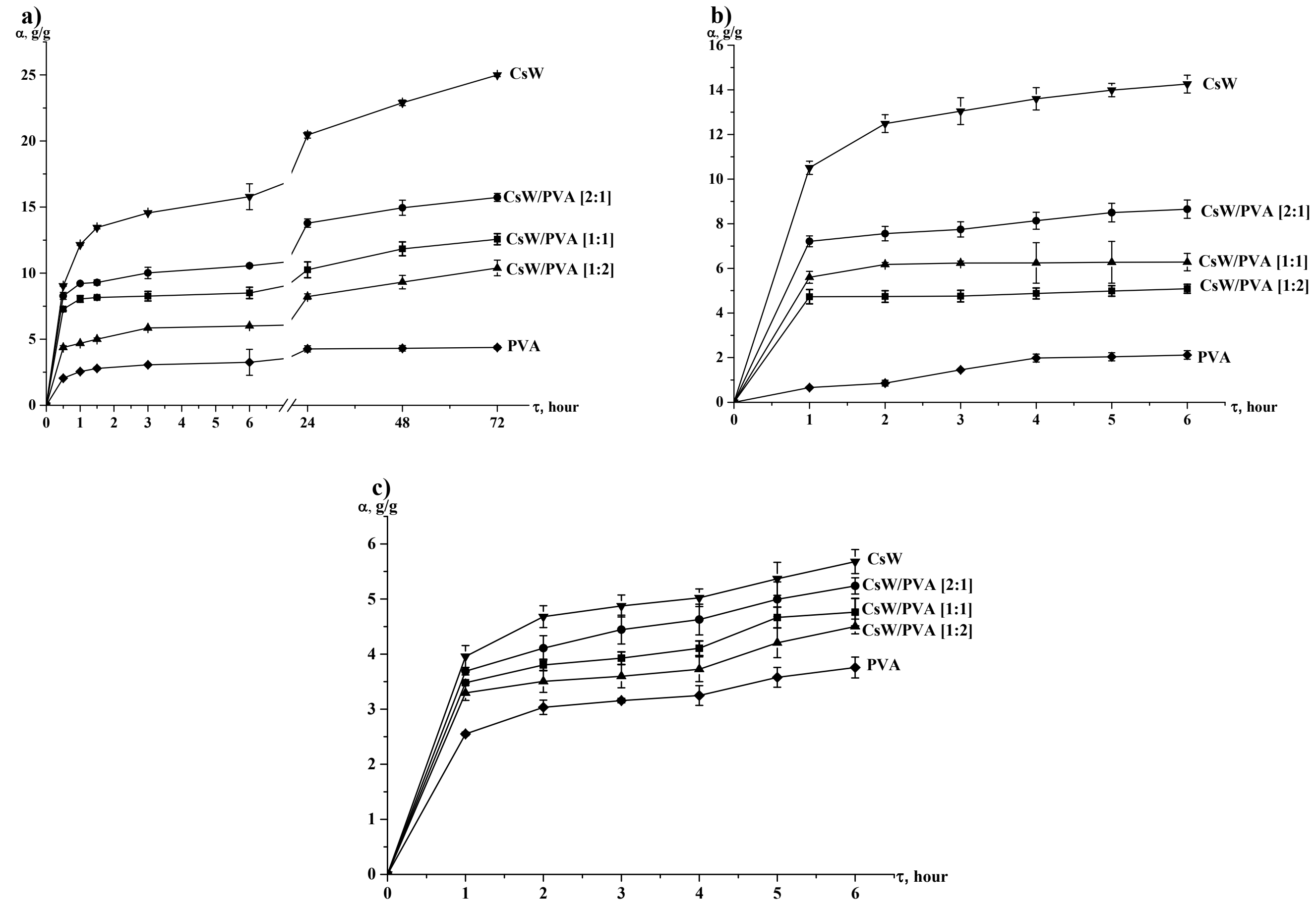


Fig. 3. The graph of swelling kinetics of CsW/PVA films: a) in water; b) at pH 3; c) at pH 9.

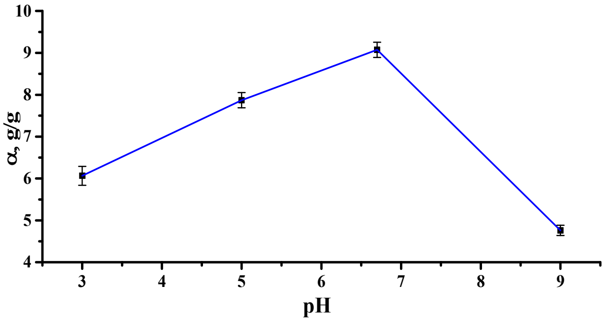


Fig. 4. Effect of pH on swelling degree of CsW/PVA film [1:1].

Comparing the swelling behavior between chitosan and PVA in water and at pH 3 and pH 9, the following order has been established: CsW > CsW:PVA [2:1] > CsW:PVA [1:1] > CsW:PVA [1:2] > PVA that also corresponds to the swelling analysis of Kulish E.I. et al. research [22]. The swelling of pure CsW in 24 h was α = 20.45 g/g, and the swelling CsW/PVA composite film with a two-fold amount of PVA was α = 7.62 g/g. According to the data, the swelling degree of films decreases with increasing the ionic concentration in the films. In addition, to determine the mass degradation the films were left until the mass disintegration in water and it showed the films in 5 days, which can be explained by the physical cross-linkage of films.

To evaluate the behavior of films in different mediums the swelling degree of films at pH 3, 5 and pH 9 (Fig. 4) was studied. The swelling degree of composite CsW/PVA with the equal ratio in 6 h was α = 3.96 g/g at pH 3 and α = 4.85 g/g at pH 9. Thus at acidic condition chitosan based films swells more than alkaline due to the ionization of the amine groups in the chitosan molecules to ammonium ion (NH_3^+) in acidic aqueous media. These cationic charges in the film phase act as cationic repulsive forces between polymer molecules [22]. Thus, this causes the dissociation of secondary interactions such as intramolecular hydrogen bonding, allowing more water into the film network.

The swelling ability of correlation CsW/BC films exceeded the swelling ability of BC film itself as shown in Fig. 5. Based on comparing the

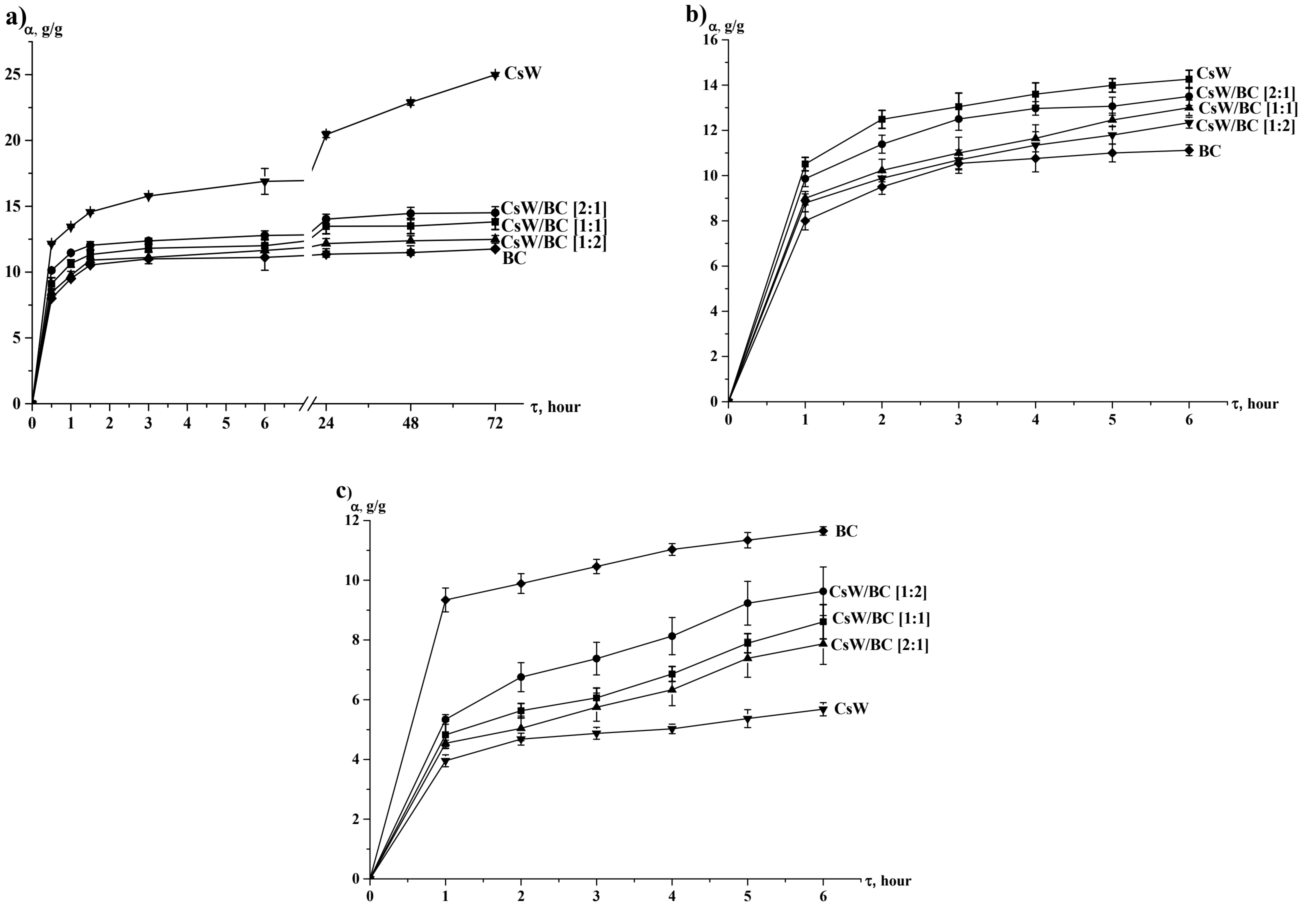


Fig. 5. Swelling kinetics of CsW/BC films a) in water; b) at pH 3; c) at pH 9.

swelling behavior between bentonite clay and CsW in water the following order has been established: CsW > CsW:BC [2:1] >CsW:BC [1:1] > CsW:BC [1:2] > BC. The swelling degree of the composite film with an equal ratio of CsW/BC in 24 h in water was α = 13.48 g/g, and the swelling of pure BC was α = 11.36 g/g. Moreover, the films after 4 days started degrading which indicates a weak interaction between the clay and CsW.

Swelling kinetics at acidic and alkaline conditions of films were different (Fig. 5 b, c). According to the results, films with two-fold amounts of BC at high pH swelled more than at low pH. As the acids replace the commonly found exchangeable cations with hydrogen ions. The dominant sodium cations (Na^+) in bentonite are replaced by hydrogen (H^+) ions. The swelling due to Na^+ as exchangeable cations is much greater than due to H^+ because of the larger ionic radius of the hydrated Na^+ ions [23, 24]. This process leads to a decrease in the swelling in the acid solution. Figure 6 shows the effect of pH on the swelling degree of CsW/BC film [1:1]. Accordingly, the swelling degree of composite CsW/BC with the equal ratio in 6 h at pH 3 was α = 13 g/g and at pH 9 it was α = 8.61 g/g.

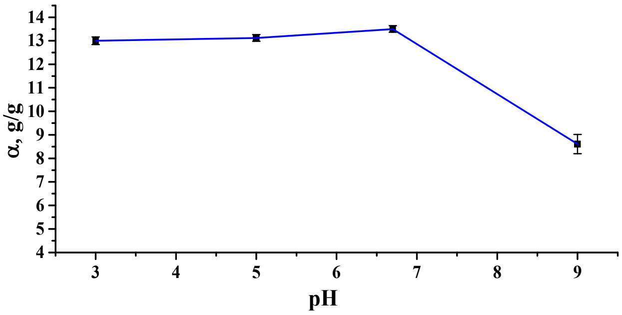


Fig. 6. Effect of pH on swelling degree of CsW/BC film [1:1].

The strength of films was compared by the tensile strength analysis in which the tensile strength was used in a chart form for comparison of the CsW/PVA and CsW/BC films (Fig. 7). Moreover, loading PVA and BC to the CsW film significantly affected the strength of the films. As the analysis shows, the tensile strength of CsW/PVA films increases with the rise of the PVA content in the film. However, the strongest one was the CsW/PVA with an equal ratio. It can be explained by the

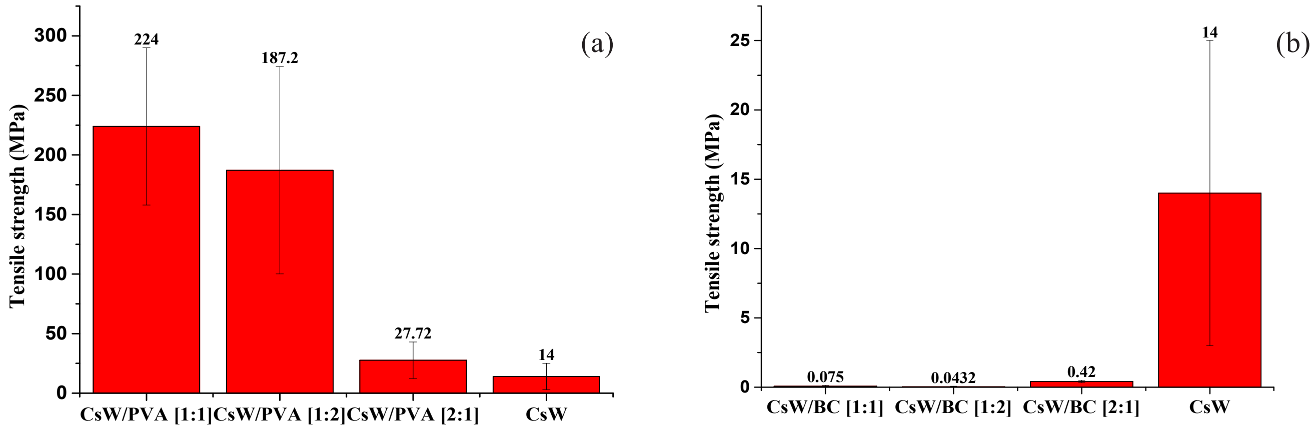


Fig. 7. Tensile strength of pure CsWs and their composite films with PVA (a) and BC (b) with different content.

strong hydrogen bonds between chitosan nanowhisker and polyvinyl alcohol. Thus, the introduction of PVA allowed us to obtain strong and elastic films at the same time.

Regarding to CsW/BC film, the tensile strength of CsW film was higher than that of composite films. Accordingly, the tensile strength of composite films was decreased with increase of BC content in the film. BC loading to the chitosan film was not observed to give the strength.

4. Conclusion

Chitosan nanowhiskers and biocomposite polymers based on CsW with PVA and BC were synthesized by a top-down approach. The interaction of CsW with PVA and BC was demonstrated by IR spectroscopy, which revealed the hydrogen bond between hydroxyl groups of CsW and PVA, and the interaction of BC with CsW by the intercalation process.

The swelling kinetics of composite films in water and various mediums was determined, as well as pure CsW, PVA, and BC. According to the results, CsW swells in water at a range from 14 to 25, PVA from 2 to 4, and BC from 8 to 11. Moreover, the composite CsW/PVA and CsW/BC films were observed to swell more in acidic conditions than in alkaline conditions. According to the results, CsW was shown to have a high swelling ability than PVA and BC.

The tensile strength of each film based on CsW/PVA and CsW/BC was studied and compared with pure CsW. The strongest film was found for the CsW/ PVA ratio of [1:1]. As for CsW/BC composite film, the CsW/BC composite with a two-fold amount of CsW was the strongest. It was observed PVA gives strength to the CsW composite material. Obtained films based on polyvinyl alcohol and bentonite clay with chitosan can be recommended for use as a wound dressing material. Further work is needed to identify studies of bactericidal activity, the degree of degradation, and the mucoadhesive properties of polymers.

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