

Molecular Characteristics of Chitosan and Structure of its Films Formed from Water Solutions of Acetic and Hydrochloric Acids

Nina Belnikovich, Elena Rosova, Irina Malakhova, Valery Krasikov, Zoolsho Zoolshoev, Elena Popova, Natalia Saprykina and Galina Elyashevich*

Institute of Macromolecular Compounds, Russian Academy of Sciences, Bolshoy pr. 31, Saint-Petersburg, 199004, Russia

Abstract: Molecular characteristics of chitosan in diluted water solutions of acetic and hydrochloric acids were studied by viscometry, dynamic light scattering, and thin layer chromatography. Chitosan molecules were found to undergo destruction in the solutions of hydrochloric acid and its mixtures with acetic acid. The structure of the chitosan films cast from these solvents was studied by DSC, TGA, and SEM. As shown by electron microscopic data, all the films had an amorphous-crystalline structure but the films prepared from chitosan solutions in diluted hydrochloric acid were characterized by a higher degree of crystallinity. It was supposed that a lower molecular mass of chitosan molecules in the hydrochloric acid solutions results in more extended conformations providing a higher capability of self-organization and formation of regular supermolecular structure. The difference in crystal modification in structure of the films cast of these acids was observed. It was found that the films prepared from the mixtures of acids had a spherulite structure and the crystal modification like ones cast from hydrochloric acid, but with a lower degree of crystallinity.

Keywords: Chitosan, water solutions of acids, viscosity, destruction, mixtures of acids solutions, chitosan film structure.

INTRODUCTION

Chitosan, a natural nontoxic, biocompatible, and biodegradable polymer, is a deacetylated soluble derivative of chitin, one of the most widespread natural polymer materials. Chitosan is widely used in various fields of medicine and cosmetic industry for a drug delivery and also as wound dressings, separation membranes and suture material [1-5]. Chitosan fibers and films are formed from solutions in weakly acidic water solvents. Water solutions of hydrochloric (HCl) and acetic (CH₃COOH) acids are used most often. Chitosan solutions were extensively studied, and all the authors noted considerable differences in the behaviors of chitosan in acidic solvents. However, at present there is no consensus on the nature of the differences [5-12]. Some authors believe that they are due to the destruction of chitosan in hydrochloric acid solutions which is observed typically at high acid concentrations. According to other researchers, the probable cause of the differences is the conformational structures of chitosan molecules in the acids mentioned above [13].

The goal of our study was to determine the characteristics of chitosan macromolecules in water solutions of acetic and hydrochloric acids and their mixtures and to estimate the influence of the solvent

composition on the structure of the films formed from these solutions.

EXPERIMENTAL

Samples

The object of studies was chitosan of Aldrich Chemical Company, Inc. (USA), medium molecular weight.

Chitosan solutions were prepared in 1% water solutions of acetic acid (WSAcA), 1% water solutions of hydrochloric acid (WSHA) and also in the mixtures of WSAcA and WSHA in volume ratios of 90/10, 75/25 and 50/50, respectively. Dissolution was carried out at room temperature at constant stirring. The solutions were filtered through a Schott filter 100 and deaerated with a water jet pump.

The films were cast from 1% solutions of chitosan in these solvents on glass substrates. They were dried in a thermostat at 30°C for 2 days and then under vacuum without heating for 1 day.

Characterization of Chitosan

The intrinsic viscosity of chitosan solutions ($[\eta]$) was measured by an Ubbelohde capillary viscometer at 25°C, the solvent outflow time was ~100 s, and the measurement error was no more than 2%. The measurements were carried out in 17 hours after the beginning of the chitosan dissolution.

*Address correspondence to this author at the Institute of Macromolecular Compounds, Russian Academy of Sciences, Bolshoy pr. 31, Saint-Petersburg, 199004, Russia; Tel: +7 812 328 68 76; Fax: +7 812 328 68 76; E-mail: elya@hq.macro.ru

The hydrodynamic sizes of chitosan molecules in acid solutions were measured by using the dynamic light scattering technique. A Photocor instrument (Russia) with a Spectra-Physics He-Ne-laser as a light source (wavelength 632.8 nm) was used. The chitosan concentration was chosen to be somewhat lower than the Debye one on the basis of viscometric data.

To perform the chromatographic analysis, microcrystalline cellulose plates DC Cellulose F, Merck (Germany) and "Basic Kit for TLC" (Scientific Technical Center "Lenchrom", Russia) were used, and the mobile phase was a four-component pyridine-ethyl acetate-acetic acid - water system (5 : 5 : 1 : 3). All chemical reagents were of the CP grade and were produced by "Vekton" (Russia), Sigma "Aldrich" (USA), Merck (Germany). Detection was performed with iodine vapors. Visualization of the chromatograms was carried out at an optical "DenSkan" densitometer (Scientific Technical Center "Lenchrom", Russia). To process the chromatographic data, the "Dens" and "Multichrom-Planar" software was used.

Investigations of the Chitosan Film Structure

Calorimetric measurements (DSC) were performed by a differential scanning calorimeter (Netzsch DSC 204 F1) at a heating rate of 5 ° C/min in argon 30 ml/min. Mass of samples was 3.5 – 4.0 mg, Al pierced lid as pan was used.

Thermogravimetric analysis (TGA) was performed by a Netzsch TG 209 F1 instrument at a heating rate of 10 ° C/min in argon 40 ml/min. Mass of samples was 1.5 – 2.0 mg, type of pan – Al₂O₃, ceramic.

The film structure was studied by scanning electron microscopy (SEM) (SUPRA-55VP, ZIESS, Germany) and wide angle X-ray scattering (Bruker D8 Discover diffractometer, Germany).

RESULTS AND DISCUSSION

Characterization of Chitosan Molecules in the Solutions WSACa and WSHA

To estimate the effect of solvent quality on molecular characteristics of chitosan in water solutions of CH₃COOH, HCl, and their mixtures, the viscometric method was used because it is the highly sensitive to changes in the sizes of macromolecules.

According to the Flory theory the intrinsic viscosity $[\eta]$ is determined by the hydrodynamic volume of macromolecular coils

$$[\eta] = [\Phi_{\theta} (h_{\theta}^2)^{3/2}/M]\alpha^3 \quad (1)$$

where Φ_{θ} is the universal Flory constant and $(h_{\theta}^2)^{3/2}$ is the macromolecular coil sizes under θ conditions; α is the degree of coil swelling in the used solvent, M – molecular mass of polymer [14].

The values of $[\eta]$ for chitosan measured in WSACa and WSHA were 27.5 and 5.8 dl/g, respectively, i.e., they differed nearly in 5 times, which points to much smaller sizes of macromolecular coils in the solution of hydrochloric acid.

The hydrodynamic sizes R_h of chitosan molecules in the acid solutions used were determined by dynamic light scattering. The values of R_h measured in the range of scattering angles 45-135° were extrapolated to a zero angle. No concentration dependence of R_h was observed for dilute solutions (0.029 - 0.0075 g/dl for the chitosan solutions in WSACa). The measurements showed that these solutions contained scattering particles with large hydrodynamic sizes of about 75 - 80 nm. At the same time, the hydrodynamic sizes of macromolecules in WSHA were 27 nm, which is nearly three times as low as in WSACa.

It was also proved by this method that two types of particles are observed in WSHA: fast (with hydrodynamical radius $R_h^{fast} = 6-7$ nm) and slow (with hydrodynamical radius $R_h^{slow} = 180-200$ nm). It is possible to suppose that values $R_h^{fast} = 6 - 7$ nm characterize the macromolecules with lower molecular masses.

Thus, the results obtained by dynamic light scattering show that the coils in the solutions of HCl have much smaller sizes than in one of CH₃COOH. These results are consistent with viscometric data.

The hydrodynamic volume of macromolecules and, hence, $[\eta]$ depend not only on the polymer nature i.e. the shape and size of macrochains, but also on the thermodynamic quality of the solvent. When the solvent characteristics change, the decrease in $[\eta]$ can be caused by both the difference in the polymer chain conformations in different solvents and destructive processes in the polymer solution in one of the solvents which lead to a polymerization degree decrease.

In our case we cannot exclude both mechanisms. In contrast to the chitosan solutions in WSACa, its solutions in WSHA contain a large number of counter ions that suppress polyelectrolytic swelling of chitosan, which should affect the sizes of polymer coils. On the

other hand, an increase in the HCl concentration leads to chitosan destruction ("fragmentation") and formation of lower-molecular mass fragments, down to oligomers, in the 1 M HCl solution [10].

To establish whether a reduction in the hydrodynamic sizes detected by viscometry and dynamic light scattering is caused by destruction of chitosan chains in WSHA, the following experiment was carried out. The film was cast from 1% solution of chitosan in WSHA. It was carefully washed from acid residues, dried to a constant mass, and then was redissolved in WSACa up to the same concentration of chitosan (1%). After that intrinsic viscosity $[\eta]$ of the chitosan solution was measured. The absence of HCl traces in the sample was confirmed by elemental analysis. The value of $[\eta]$ equal to 6.24 dl/g differed only slightly from its value in the initial chitosan solution in WSHA (5.8 dl/g) and was more than in 4 times lower than $[\eta]$ of the polymer initial solution in WSACa. Such a large fall of the polymer viscosity in the same solvent unambiguously indicated that destructive processes leading to a decrease in $[\eta]$ i.e. reduction in molecular mass of chitosan in the HCl solutions.

Chromatography allows to separate and identify complex multicomponent mixtures of substances and also simultaneously to determine the amount of each component [15]. Thin layer (planar) chromatography (TLC), a kind of liquid chromatography, is preferred in some cases because it allows detection of sorbed components of the multicomponent mixture to be analyzed in the starting zone of the TLC plate.

The TLC method was used to carry out a comparative evaluation of chromatographic activity of chitosan solutions in WSHA and WSACa (Figure 1). The chromatographic mobility of sorbates was estimated by the parameter $R_f = X/Y$ (where X is the distance from the start at which the spot travel and Y is the distance from the start to the solvent front).

Analysis of the TLC data (Figure 1) showed that in both cases chitosan is irreversibly adsorbed in the starting chromatographic zone ($R_f = 0.00$). The chromatogram of the acetic chitosan solution contains only one spot which is located in the starting zone ($R_f = 0.00$). The sample dissolved in WSHA is partially destructed and forms two more components (compounds) having mobility coefficients $R_f = 0.45$ and $R_f = 0.95$, which indicates that two components with lower molecular mass are present in addition to the initial one. A quantitative treatment of the

chromatogram at a densitometer showed that the ratio between the mixture components in the HCl solution was 6% (zone with $R_f = 0.95$), 52% (zone with $R_f = 0.45$) and 42% (zone with $R_f = 0.00$).

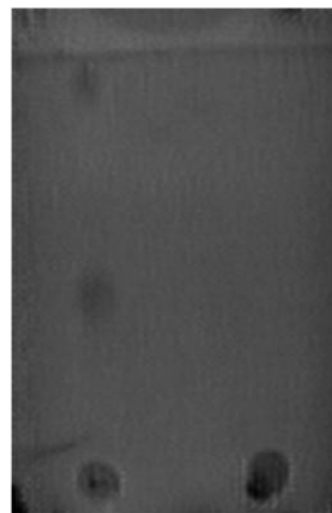


Figure 1: Thin layer chromatography of 1% chitosan solutions in WSHA (1) and in WSACa (2). Eluent: pyridine-ethyl acetate-acetic acid - water (5: 5: 1: 3). Detection: iodine vapors. TLC plates: DC Cellulose F.

Thus, the results of dynamic light scattering, viscometric, and chromatographic experiments point to the presence of a partial destruction of chitosan in the HCl solution. Note that a sharp drop in the intrinsic viscosity $[\eta]$ of chitosan at dissolution in strong acids, including HCl, was also observed by other investigators [10].

During further storage of chitosan solutions in WSACa and WSHA a slight decrease in $[\eta]$ is observed. However, these changes do not exceed 10% and are the same for both solvents, which is consistent with the data of other authors [10, 12].

By using the known value of the constants for chitosan with a deacetylation degree of 80 – 75 ($K = 1.59 \cdot 10^{-4}$ and $a = 0.79$) in Mark - Kuhn – Houwink equation $[\eta] = K(M_n^a)$, we determined viscosity-average molecular masses M_n of the initial chitosan and of the sample obtained after dissolution in WSHA and complete removal of the solvent from it [10]. The values of $[\eta]$ were measured in water solutions containing 0.25N CH_3COOH and 0.25N sodium acetate at a temperature of 25°C. The calculations showed that after dissolution in WSHA the molecular mass of chitosan decreased in 7.9 times as compared with the initial one, from 250000 to 31600 g/mol. The latter value corresponds to the degree of chitosan polymerization close to 200. This means that the

destruction occurring in HCl solutions does not lead to a complete degradation of the macromolecular chains of chitosan up to monomers, and only a partial destruction with the formation of a polymer with a lower molecular mass is observed.

Chitosan in Mixtures of WSACa and WSHA

To estimate the influence of HCl on the chitosan destruction degree the magnitudes of intrinsic viscosity for the 1% chitosan solutions in the mixtures of WSACa and WSHA in volume ratios 90/10, 75/25 and 50/50 were measured and presented at Figure 2 as $[\eta]_m^* = [\eta]_{\text{mixture}}/[\eta]_{\text{WSACa}}$ where $[\eta]_{\text{mixture}}$ and $[\eta]_{\text{WSACa}}$ are intrinsic viscosities for the chitosan solutions in the mixtures of solvents and in WSACa, respectively.

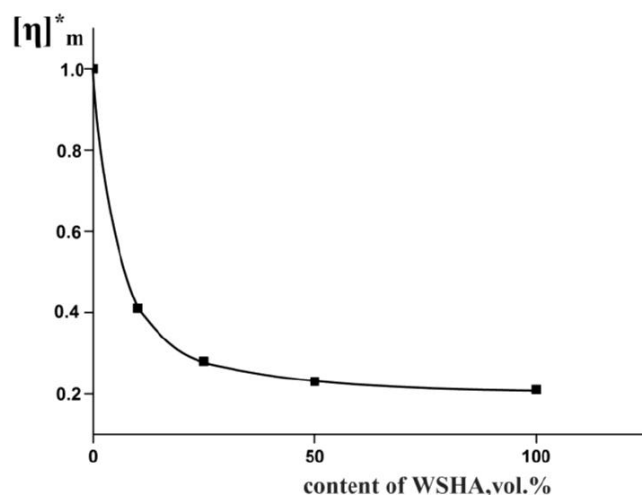


Figure 2: Dependence of $[\eta]_m^*$ on content of WSHA (vol %) in the mixture of solvents.

It can be seen from the graph that $[\eta]_m^*$ steadily decreases with increasing WSHA content in the solvent. A sharp drop is observed already at low contents of WSHA - for the mixture 90/10 $[\eta]_m^*$ decreases by 60%. A further increase in the WSHA content leads to a gradually slowing decrease in $[\eta]_m^*$. Thus, even small additions of HCl cause significant chitosan destruction in the solutions.

In analyzing the results of viscometer measurements, the hypothesis was put forward that destructive processes are developed during the chitosan dissolution. To see whether this hypothesis was correct or not, we obtained dependences of the rate of decrease in viscosity in mixed solvents on the hydrochloric acid content. The relative viscosity η_{rel} (η_{rel} was defined as the ratio of the solution outflow time to the solvent outflow time) in 0.06% solutions of chitosan in the solvents with the compositions mentioned above

was measured as a function of time. In WSACa η_{rel} did not change in the range considered here. HCl was added to the viscometer immediately at the start of the experiment. As can be seen from Figure 3, the initial values of the relative viscosity decrease with increasing HCl content. Figure 3 also shows that the decrease in the viscosity of the solutions takes place only during the first minutes of measurements, and the higher the HCl content in the solvent, the lower the level to which viscosity falls. The decrease in viscosity during the first day was of the order of 1.5% for 90/10 and 75/25 mixtures, and the decrease in η_{rel} was 6% when content of WSHA in the mixture increased up to 50/50.

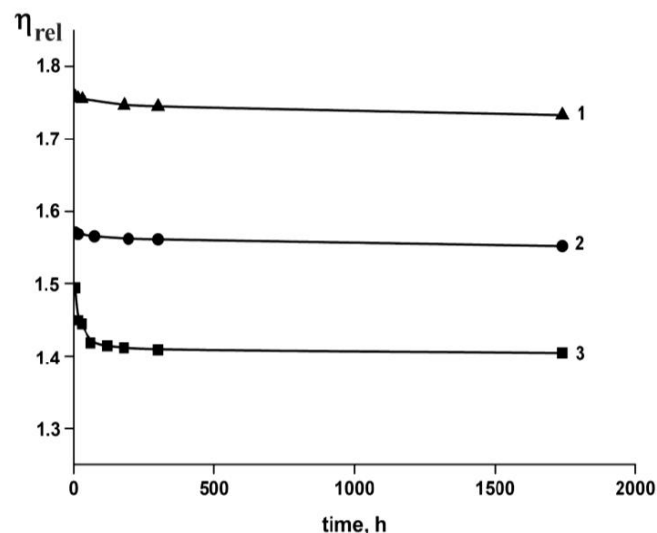


Figure 3: Time changes in relative viscosity of the chitosan solutions in the mixtures of solvents of different compositions: 1 - 90/10; 2 - 75/25; 3 - 50/50.

Structure of Chitosan Films Cast from WSACa and WSHA and their Mixtures

To study the structure of chitosan films cast from solvents of different compositions, DSC, TLC, TGA, SEM, and X-ray scattering were used. The DSC measurements (Figure 4) showed that the chitosan curves for the films cast from WSACa did not exhibit pronounced endo- or exoeffects. Only a small deviation from the linearity at temperatures above 140 °C was observed. At the same time, the DSC curves for the films cast from WSHA solutions and dried under vacuum during 5 days exhibited a slight endoeffect at 200 °C, which is in the region of thermal degradation of chitosan in the films, 170-200 °C, as it is shown by TGA (Figure 5).

The DSC data are confirmed by the results of TGA studies of sample mass losses. The start of the chitosan destruction in the films obtained from WSACa

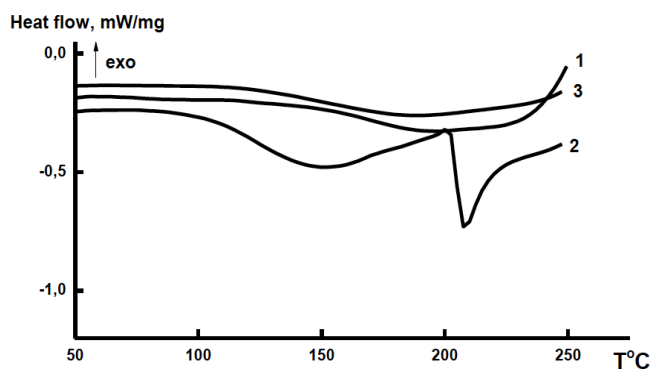


Figure 4: DSC curves of the samples: 1- chitosan film cast from WSAcA; 2 - chitosan film cast from WSHA; 3 – chitosan film cast from mixture of solvents (WSAcA /WSHA = 50/50).

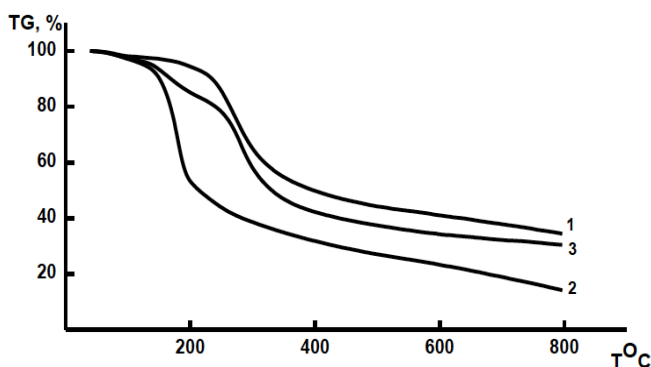


Figure 5: TGA curves of the samples: 1- chitosan film obtained from WSAcA; 2 - chitosan film cast from WSHA; 3 – chitosan film cast from mixture of solvents (WSAcA/WSHA = 50/50).

is observed at 190-200^oC, while the process of mass losses in the chitosan films cast from WSHA starts much earlier, at 110-120^oC. It should also be noted that there is a significant difference in the thermal stability of the chitosan films cast from solutions of different acids. For the sample obtained from the solution in CH₃COOH a 30% mass loss occurs at 290^oC and for the sample from the HCl solution it occurs already at 190^oC. The reduction in the sample molecular mass leads to a decrease in the temperature at which thermal destruction starts and to an increase in the mass losses from 70 to 90% for the films cast from WSAcA and WSHA, respectively.

These data agree well with the above conclusion that a partial destruction of chitosan occurs in HCl solutions and with the results presented in the previous section. As it is seen at Figures 4 and 5 the curves DSC and TGA for the films cast from mixture of solvents (curves 3) are close to ones for the samples formed from WSAcA. Figure 5 shows also that temperature range of mass losses for films from the mixture is shifted to higher temperatures as compared

with the films from WSHA. No thermal effects were observed on DSC curves (Figure 4) for the films from the mixture as for the films from WSAcA.

Since the chitosan melting point is higher than its destruction temperature, the DSC method (and other thermal methods) does not give information on its crystal structure [12]. The supermolecular structure of chitosan films was studied by SEM and wide-angle X-ray scattering techniques. Figure 6 shows electron micrographs of the surface of the chitosan films cast from WSAcA, WSHA and their 50/50 mixture. It is obvious from Figure 6a that the film obtained from the CH₃COOH solution is nearly homogeneous. Elements of structures (crystallites) have dimensions of the order of 10-20 nm, so this film is transparent in the visible light. The surface of the film obtained from the HCl solution (Figure 6b and c) has a different morphology; it contains typical spherulites formed by microfibrils. The spherulite size is 1.2-1.5 μm, the microfibril diameter is 20-25 nm and length is 400-600 nm. The film is nonhomogeneous in structure and comprises transparent (Figure 6b) and opaque (Figure 6c) areas, which points to phase separation caused by the polymer concentration increase during sample drying. The picture Figure 6c demonstrates the spherulites which form dense grains of the crystalline phase in the amorphous medium. The presence of interfaces between these phases leads to light scattering and opacity of the films.

Figure 6d, e present a micrographs of the film surface obtained from the solution of the mixture of WSAcA and WSHA. The morphological picture is similar to that for the samples cast from the HCl solution: there are grains of spherulites formed by fibrils, but in this case the picture is less sharp, spherulites are less perfect, and the sample is more amorphous. The micrographs taken at a smaller magnification point to a homogeneity of the structure of these samples and the presence of discrete structural elements.

The X-ray scattering data shown in Figure 7 indicate that all the films have an amorphous-crystalline structure, but the samples cast from chitosan solutions in WSAcA (curve 1) have a lower degree of crystallinity than the films from solutions in WSHA (curve 3): ~8 and ~29%, respectively. There is also a difference in their crystal modification (shifting of crystalline peaks). The crystalline modification of the film from a mixed solvent (50/50) (curve 2) corresponds to the modification of the structure of the samples cast from

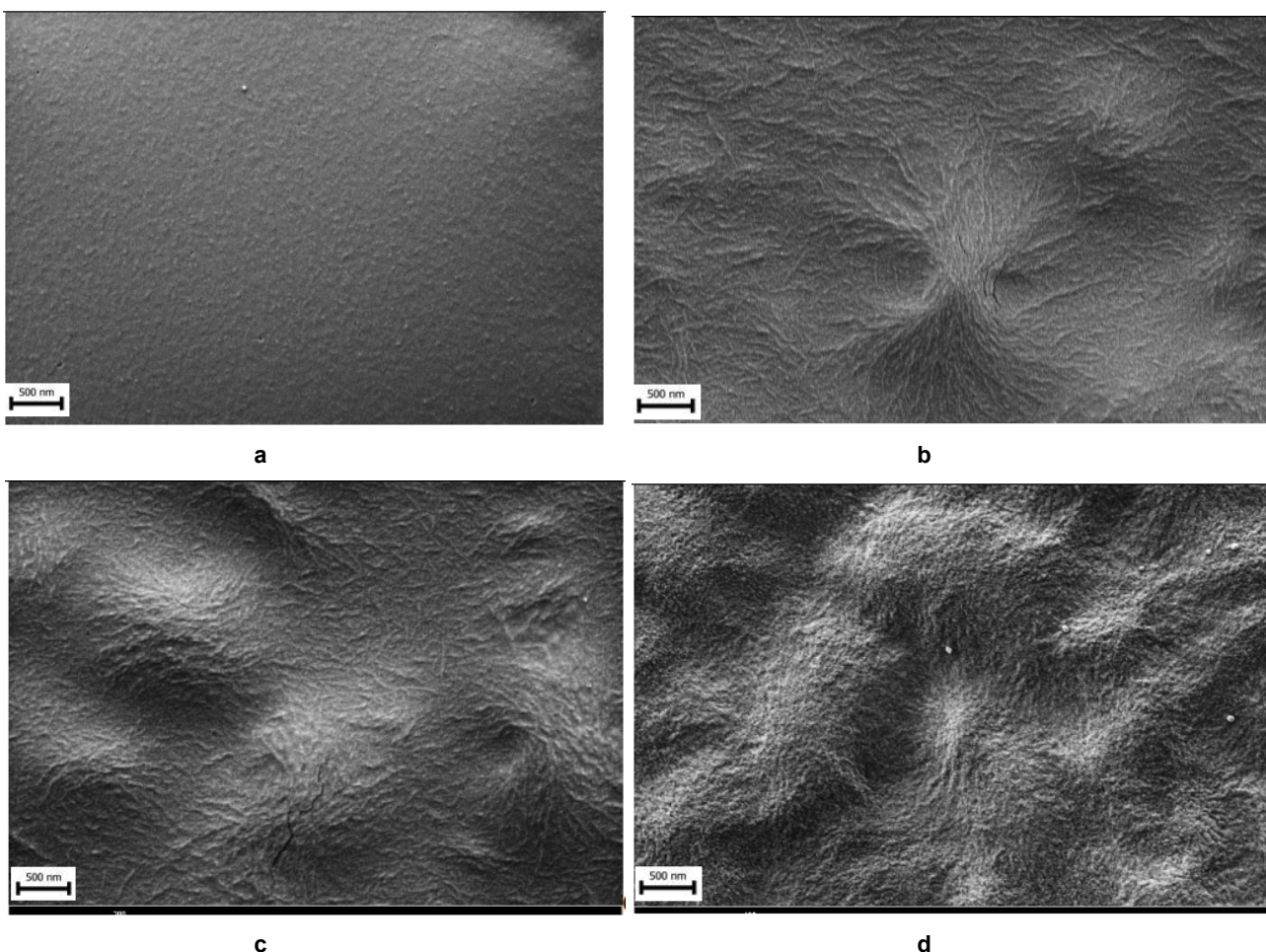


Figure 6: Electron micrographs of surfaces of the films cast from chitosan solutions in WSAcA (a), WSHA (b, c) and their mixture 50/50 (d).

solutions in WSHA, but the crystallinity in this case is slightly lower (~24%).

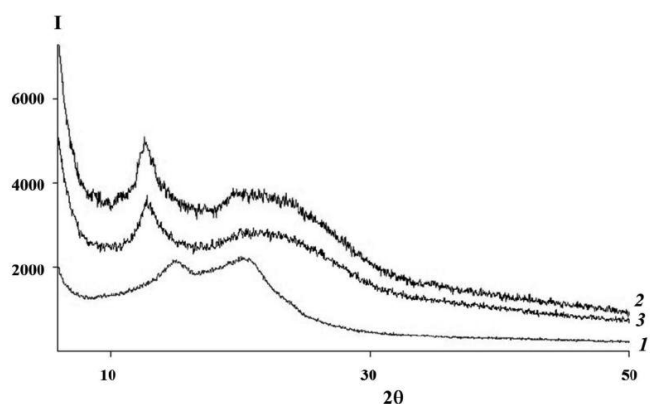


Figure 7: Wide-angle X-ray curves for the films cast from chitosan solutions: 1 - in WSAcA; 2 - in WSHA; 3 - in mixture WSAcA/WSHA = 50/50.

It can be supposed that the ordered packing of chitosan macromolecules in the film cast from the solution in CH_3COOH is not realized because of a larger length of molecular chains and a greater number

of intra- and intermolecular contacts than in the films from HCl solution. This leads to the formation of nearly amorphous (the degree of crystallinity is ~8%) homogeneous sample. The film obtained from solution in WSHA contains the macromolecules of a lower molecular mass because of the partial chitosan destruction and a rupture of intermolecular bonds in this solution. The shortened macromolecular chains acquire a higher capability of self-organization and, hence, a higher crystallinity and the more ordered supermolecular structure.

CONCLUSION

To summarize, it was found that the chitosan dissolution in the diluted solutions of HCl leads to a partial destruction of its chains, which manifests itself in a sharp decrease in the intrinsic viscosity as compared with that for the diluted solutions of CH_3COOH . This effect occurs also in mixtures of water acid solutions and becomes stronger as the HCl content increases. The studies showed that the chitosan films cast from

the solutions WSAC and WSHA have different structures. At the same time, the films from mixtures of acid solutions have the structure similar to that of the films from WSHA, but exhibit a lower degree of crystallinity. It is known that the macromolecular coils with a lower molecular mass have a more elongated conformation and consequently more readily form regular spherulite (or domain) structures. The data obtained in our research allowed us to establish the influence of molecular characteristics of chitosan in dilute CH₃COOH and HCl solutions on the self-organization processes at supermolecular structure formation and degree of homogeneity of the films prepared from these solutions and their mixtures.

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