New aspects of thermal treatment effects on gelatin films studied by microhardness

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SUMMARY:

Microhardness measurements were carried out using various treatment cycles, including heating and cooling during different treatment times at high temperatures. Two possible processes to explain the observed increase in the microhardness are proposed, namely crystallisation and crosslinking. In order to distinguish between these two alternatives, DSC, WAXS and swelling kinetics measurements were performed. The observed decrease of crystallinity (from DSC and WAXS measurements) as well as the decrease of swelling ability with increasing temperature and duration of thermal treatment are in favour of the occurrence of crosslinking reactions during thermal treatment. It is suggested that the crosslinking, as a result of additional intra- and intermolecular condensation processes, leads to a denser chain packing in the amorphous gelatin and consequently to higher microhardness values.

Introduction

In a preceding study, we reported¹⁾ on unusually high surface microhardness (H) of gelatin films after thermal treatment. Even thermally untreated gelatin films show a surprisingly high surface microhardness, as compared to that of synthetic polymers²⁾. Room conditioned gelatin films, containing about 15 wt-% water - the latter having a strong plasticizing effect and thus causing a significant softening of the material³⁾ - has a microhardness value of about 200 MPa²⁾. After drying, H increases up to 390 MPa in the temperature range 135-160°C²⁾. It is worth mentioning here that paraffins, polyethylene (PE) and metals, such as Pb and Sn, exhibit microhardness values below 100 MPa; semicrystalline poly(oxymethylene), poly(ethylene terephthalate), extended-chain PE, poly(ethylene 2,6-naphthalate) and metals, such as Al, Au, Ag, Cu and Pt, show values between 100 and 300 MPa; carbon fibre-reinforced polymer composites - 900 MPa and common metals, such as Zn and Co - 2000 and 4000 MPa, respectively, white steel - 5000 MPa⁴⁾. Thermally untreated gelatin with its microhardness of 400 MPa really surpasses most commonly used synthetic polymers and soft metals, and the thermally treated polymer, having a microhardness of almost 700 MPa¹⁾, approaches the hardness values of carbon fibre-reinforced composites.

The observed unusual increase in the microhardness of gelatin films after thermal treatment was explained by the chemical peculiarities of this polymer¹⁾. Being a polypeptide, in contrast to polyamides, that are chemically close to it, gelatin is characterized by the presence of a large amount of free

side-chain carboxyl, hydroxyl and amine groups, arising from the diaminomonomocarboxylic and monoaminodicarboxylic acids. On the other hand, condensation polymers are well known to undergo additional condensation^{5,6}, involving the end groups when appropriate conditions are available (temperature, catalysts and vacuum are the major factors accelerating the process⁶). Similar interactions between reactive side-chain groups can be expected in the case of proteins^{7,8}. For instance, the insolubility of gelatin after sufficiently prolonged evacuation (days!) at a temperature between 65 and 105°C is explained by the formation of a three-dimensional network, resulting from interchain crosslinking⁷. This conclusion is supported by the fact that such an insolubility is not observed with chemically modified gelatin (by acetylation of the amino groups or by esterification of the carboxyl groups⁹). Finally, swelling ratio measurements of thermally untreated and treated gelatin as well as the change in the aggregative state of the annealed sample¹ recently led to the same conclusion. It was assumed¹ that as a result of chemical links formation between chain segments, regardless of whether intra- or intermolecular, a denser chain packing was achieved. Such a densification ought to lead to increased microhardness, since it is known that these two properties are closely related^{4,10}.

The purpose of this study is to provide a deeper insight to the effect of thermal treatment conditions on the microhardness of the gelatin films. To this end, microhardness measurements were carried out using various treatment cycles, including heating and cooling during different treatment times at high temperatures. The microhardness evaluation was selected as a test method for

the surface characterization because of its simplicity and high sensitivity. It is known that this method gives useful information about the microstructure of polymers, provided that relationships between microhardness and crystallinity, crystallite perfection, chain conformation and other structural parameters have been derived^{4,10,11}.

Experimental part

Materials and sample preparation

Gelatin powder (type A: from porcine skin, 300 bloom) was purchased from Sigma. Films were prepared from gelatin powder soaked overnight in distilled water at 5°C, then dispersed by the addition of some water at 50°C (water bath) and cast in an aluminium Petri dish. An isotropic gelatin film with a moisture content of about 15-17 wt-% was obtained after drying under room conditions for 2-3 days, and will be further designated as room-conditioned sample (Sample 3).

Samples for microhardness

A room-conditioned gelatin film was dried for 5 h at 140°C under vacuum. Then a piece from the dry film was moistened up to 11 wt-% water content by placing it for a few minutes between two wet filter papers in order to depress the glass transition temperature. The moistened gelatin was inserted in a sealed beaker containing some water at the bottom. The beaker was placed into

an oven at 90°C for 5 h till crystallization took place. Thereafter, the sample was dried at 80°C for 5 h under vacuum (Sample 1 in Table 1).

Completely dry gelatin samples used to follow the time dependence of microhardness at high temperature were prepared by placing the room-conditioned gelatin film in an oven for 22 h at 105°C and then under vacuum for 41 more hours at the same temperature (Sample 2 in Table 1). According to Yannas and Tobolsky⁷⁾, after such a treatment the sample weight reaches a constant level ($\pm 0.05\%$).

Samples for swelling kinetics and WAXS

The room-conditioned gelatin film (Sample 3 in Table 1) was cut into 1 cm² square pieces. Three of these pieces were used to obtain the standard swelling kinetics curve, and the fourth one to obtain the native gelatin diffractogram. The other pieces were treated in the same way as sample 1, i.e. dried at 140°C for 5 h under vacuum, moistened up to 11 wt-% water content, annealed at 90°C for 5 h in water vapour atmosphere and dried again at 80°C for 5 h under vacuum. After this treatment, halves of these pieces were placed on a heating plate at 100°C for 10 minutes (Sample 4). This was done to simulate the conditions at the beginning of the temperature dependence measurement of microhardness. The remaining halves of the treated pieces were placed on the heating plate at 100°C for 20 min, then the temperature was raised up to 123°C and maintained for 20 min. Thereafter, the samples were kept for 30 min at 146°C, for 30 min at 168°C, for 35 min at 180°C and for 10 min at 191°C (Sample 5). The final thermal treatment simulates the conditions at the

end of the microhardness temperature dependence measurement. The samples preparation conditions and their designation are summarized in Table 1. Swelling kinetics curves and WAXS diffractograms were obtained with Samples 3, 4 and 5.

Samples for DSC

Samples for DSC measurements were prepared in the same way as samples 3 and 4 (see Table 1), however, for the last sample the non-isothermal treatment was performed in the DSC cell rather than on the heating plate. Immediately after reaching the highest temperature of the non-isothermal treatment (100°C), DSC thermogram was taken. Therefore the thermogram of Sample 4 begins at 100°C.

Techniques

Microhardness was measured at elevated temperatures, using a Leitz tester equipped with a square-based diamond indenter in conjunction with a hot stage. The microhardness value (in MPa) was derived from the residual projected area of indentation according to the expression⁴⁾:

$$H = k \frac{P}{d^2} \quad (1)$$

where d is the length of the impression diagonal in meters, P the contact load applied in N and k is a geometric factor equal to 1.854. A loading cycle of 0.1 min and a load of 2N were used. Ten measurements were averaged for each point. Since the values of T_g and T_m of dry gelatin obtained by microhardness tests differed from the reported ones³⁾ as well as from those derived by DSC

using the same samples¹²⁾, a calibration of the hot stage temperature was performed using crystals of seven organic compounds covering the temperature range of interest (70-210°C). The test substances were placed on the surface of the gelatin film where microhardness was measured. A fairly good linear correlation was achieved. The microhardness values of the gelatin films were determined as follows: each sample was placed on the hot stage of a Leitz tester at 100°C and 10 indentations were performed. The temperature was raised to 123°C (for 20 min) and 10 further indentations were made. The microhardness of the gelatin sample was similarly measured at 146, 168, 180 and 191°C respectively. Then microhardness measurements during cooling in order of decreasing temperature were made (from 180 to 100°C). Thereafter the next heating-cooling cycle was performed. Between each microhardness determination cycle the sample was kept into a desiccator. Four cycles were performed, each time reaching a higher upper temperature - 191, 205, 214 and 261°C, respectively.

Each sample, prepared for swelling measurements, was immersed in 50 ml of distilled water at 20°C. Over a period of 100 minutes, at every 5 minutes, the sample was weighed after gentle surface wiping, using lint-free tissues and returned to the swelling water. The swelling ratio S was calculated using the following equation¹³⁾:

$$S = \frac{W_s - W_i}{W_i} \quad (2)$$

where W_i is the initial weight of the sample, W_s is the weight of the swollen sample at immersion time t . The swelling measurements were performed for three samples of each type and the data were averaged.

The heat of fusion and the melting temperature of the samples were determined using a Mettler TA-3000 differential scanning calorimeter in the temperature range of 40 - 260°C at a heating rate of 10°C/min.

Wide-angle X-ray scattering (WAXS) patterns of gelatin samples with different thermal prehistory were recorded at room temperature on a standard TUR M 62 diffractometer using CoK α radiation in the transmission mode.

Results

Temperature and heat treatment time dependance of microhardness

Fig. 1 shows the nearly linear microhardness increase with temperature observed for a dried gelatin film (Sample 2) from 100 to 191°C. In a preceding study²⁾, we demonstrated that room-conditioned, i.e., non-dried gelatin exhibits a microhardness value of about 200 MPa, which increases with the temperature rise (to 225°C) during measurement up to 400 MPa. Hence the relatively high initial value (437 MPa) obtained in the present case, is consistent with the microhardness value of dried gelatin films.

In the present study the thermal treatment of the sample used during the microhardness measurements has two peculiarities: (i) it is non-isothermal and, what is more important, (ii) it is relatively short (20-30 min at each temperature), in contrast to the subsequent measurement at constant temperature. For this reason, an attempt was made to follow the microhardness behaviour of gelatin (Sample 2 in Table 1) at the highest temperature (191°C) for a longer time interval (33 hours). The results are plotted in Fig. 2.

Measurements were performed every 5 min, and the values of the two impression diagonal lengths were averaged. The kinetic nature of the experiment did not allow to take more measurements at a given constant set of conditions. The relatively low accuracy of each point in Fig. 2 taken at a single set of conditions is compensated by the very large number of measurements. Regardless of the large scattering of data, one sees during the first 400 min a strong H increase (from 520 up to 670 MPa) of the gelatin film. Longer treatment times result in a sharp drop (almost to the initial value, i.e., to about 550 MPa). After 420 min there is a slight H variation in this value, and only after treatment times longer than 1200 min a tendency to decrease is observed.

The same sample used to follow the time dependence of the microhardness at high temperature (191°C) for 2000 min (Sample 2 in Table 1), was subjected to non-isothermal cooling down to 146°C, heated again up to 246°C and finally cooled to 100°C. During the above thermal treatment indentations were performed in order to obtain the temperature dependence of microhardness. Results are plotted in Fig. 3. One can observe that the first cooling (from 191°C to 146°C) does not change significantly the microhardness values (about 512 MPa) (Fig. 3a). However, the subsequent temperature rise (up to ca. 214°C) causes a slight decrease of microhardness, followed by a similar microhardness increase up to 246°C (Fig. 3b). A detectable change in microhardness takes place during the cooling from 246°C to 100°C. During the first 50-60°C the microhardness drops by 25% (from 554 to 418 MPa), keeping a value of 440 MPa down to 100 °C (Fig. 3c).

Finally, the temperature dependence of a dry gelatin film (Sample 1, Table 1) was followed in various heating-cooling cycles by increasing the upper limit temperature after each cycle. The results are displayed in Fig. 4.

The well established H increase during the first heating up from 100 to 191°C (Fig. 1) is again observed (Fig. 4a). After cooling down to 100°C the microhardness continuously increases, reaching the value of 496 MPa. During the second cycle (Fig. 4b), heating above 150°C causes a H increase up to 534 MPa when the temperature approaches 180°C, followed by an abrupt drop down to 460 MPa within 10-15°C. The last microhardness value is more or less preserved during the subsequent cooling of the sample down to 100°C (Fig. 4b).

During the third cycle (Fig. 4c), when the temperature reaches 214°C, the microhardness values are simply scattered between 480 and 550 MPa, this situation being dramatically changed during the last cycle (Fig. 4d): on heating, the constant value of microhardness of 545 MPa is observed up to about 168°C followed by a continuous drop till 248°C, when the microhardness reaches the value of 416 MPa. During cooling this value almost remains constant.

Both factors, temperature (Figs. 1, 3 and 4) and duration (Fig. 2) of thermal treatment affect microhardness. This is assumed¹⁾ to be due to crosslinking between free side-chain groups in the protein molecule. In order to verify this assumption, additional experiments were carried out, aiming to follow the effect of crosslinking on other gelatin properties, such as crystallinity, solubility, and degree of swelling, in addition to our preliminary swelling behavior measurements¹⁾.

Differential scanning calorimetry

Fig. 5 illustrates the DSC traces from: a) thermally untreated gelatin (Sample 3 in Table 1), and b) a thermally treated sample at 100°C for 10 min (Sample 4 in Table 1). The two thermograms show one high temperature melting peak, T_m , with close values for the two samples. In addition, both thermograms show a lower temperature melting peak T'_m with widely different values. The appearance of more than one melting peak in the thermograms of polymers is a well documented phenomenon¹⁴⁾. The most common interpretation is related with recrystallization processes during the scanning in the DSC, although other reasons, as coexistence of more than one crystalline population differing in their perfection, can lead to the same effect¹⁴⁾.

In the present case, the most probable reason for the observation of two melting peaks, as demonstrated in a previous study¹²⁾, is a recrystallization process. This means that the crystallites created before placing the sample in the DSC apparatus melt at T'_m . The second melting at T_m arises from the crystallites created during the thermal scanning. Let us take into account the special measurement conditions in this particular case, i.e. the fact that the sample 4 is directly annealed in the DSC instrument, and that the measurements start at different temperatures for the two samples (Fig. 5). For the purposes of this study the relevant values are those of T_m . The degree of crystallinity is evaluated from the area of this peak, since the supposed crosslinking during annealing will affect only T_m , but not T'_m . The values for both T_m and T'_m as well as the respective heats of fusion, giving an idea about the degree of crystallinity w_c for the two samples of Fig. 5, are summarized in Table 2. A quite interesting

tendency is observed: the thermally untreated sample (Sample 3 in Table 1, Fig. 5a) is distinguished by the lowest T_m value, but at the same time by the highest degree of crystallinity ($\Delta H = 4.06$ J/g, Table 2). The thermally treated sample (Sample 4 in Table 1, Fig. 5b) shows higher T_m and lower w_c values ($\Delta H = 2.03$ J/g, Table 2). The decrease in the degree of crystallinity could originate from crystallization hampering factors. The observed results- more perfect crystallites, as concluded from their high T_m , but decreasing in amount - can be interpreted only as an indication of intensive chemical reactions resulting in crosslinking of the samples during annealing (Sample 4 in Fig. 5 and Table 2). Thus, the restricted crystallization ability with the progress of the annealing supports the above conclusions about crosslinking due to condensation reactions.

Wide Angle X-Ray Scattering

Fig. 6 shows the WAXS diffractograms for: a) a room-conditioned gelatin film (Fig. 6a, Sample 3 in Table 1), b) a thermally treated sample at 100°C for 10 min (Fig. 6b, Sample 4 in Table 1), and c) another gelatin film thermally treated for a longer time (Fig. 6c, Sample 5 in Table 1). In the diffractogram presented in Fig. 6a, two crystalline peaks, at $2\theta = 8^\circ$ and $2\theta = 36^\circ$, and an amorphous halo, centered at $2\theta = 23.9^\circ$, can be seen. According to the recent interpretation of Itoh et al.¹⁵⁾, the peak at $2\theta = 8^\circ$ is due to the repeat of triple helical protofibril in the direction perpendicular to the fibre axis of a junction zone. This peak corresponds to a repeat distance of

12.6 Å. The second peak at $2\theta = 36^\circ$ is presumably derived from the amino-acid residues in the junction zone (crystallites) at a repeat distance of 2.9 Å¹⁵⁾.

Table 3 summarizes the lattice spacings, d , and the average “coherence length” values, D , corresponding to the “crystalline” and “amorphous” maxima for the three samples studied. It is interesting to note that the crystal size perpendicular and parallel to the chain in the room-conditioned sample (Fig. 6a) present very small values (57 and 78 Å respectively). These values are only slightly larger than the “coherence length” corresponding to the clusters of the molecules in the amorphous phase ($D \sim 16$ Å). After the heat treatment the scattering patterns of samples 4 and 5 (Fig. 6b and 6c) show the disappearance of the two crystalline peaks, with only the “amorphous” maximum remaining having a slightly larger “coherence length” of $D \sim 19$ Å. This means that, after removal of the water, the packing of the amino-acid residues and the helical arrangements of the protofibrils in the better ordered regions are lost. However, the average packing of all the molecules in the material after water removal, giving rise to a slightly higher “coherence length” $D \sim 19$ Å must be presumably responsible for the higher melting peak T'_m observed.

In addition to this, the disappearance of the two crystalline peaks after thermal treatment suggests amorphization of the gelatin samples due to crosslinking in agreement with DSC measurements (Fig. 5).

Swelling

The occurrence of a crosslinking process is also supported by the swelling experiments. The swelling curves of gelatin films, differing in their thermal prehistory, are shown in Fig. 7. The swelling of native gelatin film (Sample 3 in Table 1) (Fig. 7a) is the one showing fastest rate. With increasing time and temperature of sample treatment a decrease of the swelling ability is observed (Samples 4 and 5 in Table 1) (Fig. 7b and c). The rate of the swelling process of all samples is described as a second order process¹³⁾. The results in Fig. 7 confirm this contention. The equation describing the swelling process is¹³⁾:

$$\frac{dS}{dt} = k(S_{eq} - S)^2 \quad (3)$$

where $\frac{dS}{dt}$ is the rate of swelling at any given time t , k is a specific rate constant, S_{eq} is the equilibrium swelling ratio and S is the swelling ratio. After integration one obtains:

$$\frac{t}{S} = \frac{1}{kS_{eq}^2} + \frac{t}{S_{eq}} \quad (4)$$

Fig. 8 illustrates the linear dependence of $\frac{t}{S}$ vs. t for Samples 3, 4 and 5. This representation allows one the calculation of the specific equilibrium swelling ratio S_{eq} (Tab. 4). As it can be seen, the S_{eq} values for Samples 4 and 5 are almost equal (within the experimental error), and lower than the S_{eq} for Sample 3. One possible explanation of this finding is the occurrence of crosslinking between macromolecules during the treatment at high temperatures. These reactions lead to the formation of a network, hindering the penetration of the

solvent molecules (water in this case) and in this way the equilibrium swelling ratio S_{eq} decreases¹³⁾. On the other hand, the same treatment leads to improved microhardness of the gelatin samples which could also be explained by intermolecular crosslinking.

One can easily see that the treatment at higher temperatures (or longer times) strongly restricts the swelling ability. Such a behaviour is usually interpreted¹⁶⁾ as a result of progressive crosslinking.

Finally, the results of a solubility test of the thermally treated samples are also in favour of the occurrence of crosslinking. For instance, while thermally non-treated gelatin readily dissolves in water even below 40°C, the sample used to follow the temperature dependence of microhardness (Sample 1, Fig. 4) remains insoluble, even when left in water at 60-70°C for several hours.

Discussion

In previous studies^{1,2)}, a very strong effect of thermal treatment on the microhardness of gelatin was observed and has been confirmed more in detail in the present investigation. Both factors, the temperature (Figs. 1, 3 and 4) as well as the treatment time (Fig. 2), significantly influence the H values. The treatment duration affects microhardness up to some limit (6-7 h) and thereafter it has a negative effect - a strong drop in microhardness is observed (Fig. 2).

This effect of thermal conditions on H can be explained only by the occurrence of chemical interactions between side-chain groups of carboxyl,

hydroxyl and amino type. They are known to be readily involved mainly in condensation reactions^{5,6)}, resulting in the formation of a more or less dense three-dimensional network. The crosslinked chains or chain segments induce a denser packing of the chains. At the same time, it is well known that materials with higher density are characterized by higher microhardness, too.

In addition to the existing data on the occurrence of crosslinking⁷⁻⁹⁾, some other proofs in favour of this contention are obtained in the present results, such as a strongly reduced crystallization ability with the progress of crosslinking. This is demonstrated by thermal (DSC) (Fig. 5), WAXS (Fig. 6) and swelling experiments performed. The fact that the thermally treated gelatin films do not dissolve in water even at elevated temperature can also be regarded as evidence for the occurrence of crosslinking.

Another peculiarity of the influence of temperature on microhardness is that when the temperature reaches 180-200°C, H decreases significantly (Fig. 4b and d). This drop in H should be related to the softening of the material since the T_g of dried gelatin lies in this temperature range. In a previous study where no thermal pre-treatment was applied²⁾, a tendency of gelatin to harden with the further rise of temperature was observed. This is not the case in the present work - here a continuous decrease in the range of 180-248°C is registered (Fig. 4d), because the highly cross-linked system is not capable to crystallize anymore.

Conclusion

In conclusion, due to the additional condensation of free side-chain groups enhanced by elevated temperatures, a three-dimensional network, characterized by a denser packing which gives rise to an enhanced microhardness is formed. The measured H values of almost 700 MPa surpass those of all known synthetic polymers approaching the ones of carbon fibre-reinforced polymer composites.

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Table 1. Samples preparation conditions.

Sample	Initial water content wt-%	Drying in vacuum		Moisture content wt-%	Annealing		Drying in vacuum	
		Temp (°C)	time (h)		Temp (°C)	time (h)	Temp (°C)	time (h)
Sample 1	15-17	140	5	11	90	5	80	5
Sample 2	15-17	-	-	-	105	22	105	41
Sample 3	15-17	-	-	-	-	-	-	-
Sample 4	15-17	140	5	11	90	5	80	5
Sample 5	15-17	140	5	11	90	5	80	5

Table 1. (Continuation). Additional thermal treatment of the samples for DSC, WAXS and swelling kinetics measurements

Sample	Annealing conditions											
	Temp (°C)	time (min)	Temp (°C)	time (min)	Temp (°C)	time (min)	Temp (°C)	time (min)	Temp (°C)	time (min)	Temp (°C)	time (min)
Sample 3	-	-	-	-	-	-	-	-	-	-	-	-
Sample 4	100	10	-	-	-	-	-	-	-	-	-	-
Sample 5	100	20	123	20	146	30	168	30	180	35	191	10

Table 2. Melting temperature and heat of fusion (from DSC) for Samples 3 and 4.

Sample	Melting temperature (°C)		Heat of fusion (J/g)	
	T'_m	T_m	Δ'	Δ
Sample 3	98	221	112.12	4.06
Sample 4	163	237	177.7	2.03

Table 3. Lattice spacings (d) and coherence length (D) for the crystalline peaks and amorphous halo derived from the WAXS patterns for gelatin samples 3, 4 and 5.

Samples	Crystalline spacings (Å)		Amorphous spacing (Å)	Crystal size (Å)		Cluster size (Å)
	d ₁	d ₂	d _a	D ₁	D ₂	D _a
Sample 3	12.6	2.9	4.7	57	78	16.2
Sample 4	---	---	4.8	---	---	19.0
Sample 5	---	---	5.0	---	---	19.4

Table 4. Equilibrium swelling ratio S_{eq} and regression coefficient for Samples 3, 4 and 5.

Sample	S_{eq}	Regression coefficient
Sample 3	13.2 ± 0.4	0.99
Sample 4	9.7 ± 0.6	0.96
Sample 5	10.8 ± 0.6	0.97

Figure captions

Fig. 1. Temperature dependence of microhardness of a dry gelatin film (Sample 2 in Table 1).

Fig. 2. Time dependence of microhardness of a dry gelatin film treated at 191°C (Sample 2 in Table 1).

Fig. 3. Temperature dependence of microhardness of a dry gelatin film (Sample 2 in Table 1) heated up to 191°C and thereafter treated as follows: *a*) cooled down to 146°C; *b*) heated from 146°C up to 248°C; *c*) second cooling down to 100°C (○-heating, ●-cooling).

Fig. 4. Temperature dependence of microhardness of a dry gelatin film (Sample 1 in Table 1) in a heating-cooling regime after increasing the upper limit temperature to: *a*) 191°C; *b*) 205°C; *c*) 214°C; *d*) 248°C (○-heating, ●-cooling).

Fig. 5. DSC curves of: *a*) untreated gelatin (Sample 3 in Table 1) and after heat treatment; *b*) Sample 4 in Table 1.

Fig. 6. WAXS curves of: *a*) untreated gelatin (Sample 3 in Table 1) and after heat treatment; *b*) Sample 4 in Table 1 and *c*) Sample 5 in Table 1.

Fig. 7. Swelling curves of: *a*) untreated gelatin (Sample 3 in Table 1) and after heat treatment; *b*) Sample 4 in Table 1 and *c*) Sample 5 in Table 1.

Fig. 8. Linear dependence of the quotient: time of swelling t / swelling ratio S (t/S) vs. swelling time t for: *a*) untreated gelatin (Sample 3 in Table 1) and after heat treatment; *b*) Sample 4 in Table 1 and *c*) Sample 5 in Table 1.

Fig. 1

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8