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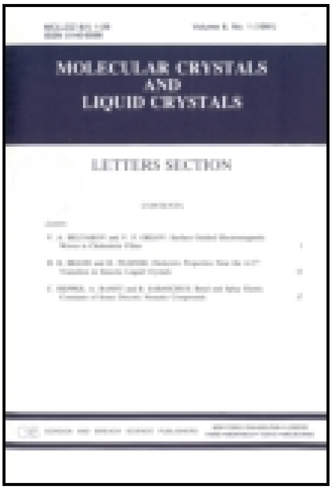

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The Processes Induced by UV Light in Biopolymers and Biopolymer Composites

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The Processes Induced by UV Light in Biopolymers and Biopolymer Composites

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Thin films by solvent evaporation from mixture of collagen, chitosan and silk fibroin were prepared. The surface properties of biopolymeric films were investigated using technique of Atomic Force Microscopy and by means of contact angle measurements allowing the calculation of surface free energy. Measurements of the contact angle on the surface were made and surface free energy was calculated. The surface of films was modified by UV-irradiation ($\lambda = 254$ nm and 248 nm). It was found that the surface roughness of biopolymeric films decreased with increasing UV-irradiation time. Polar component of the surface free energy increased with increasing UV-irradiation time.

Keywords collagen; chitosan; silk fibroin; polymer blends; surface properties; UV-irradiation

Introduction

UV-irradiation can modify several properties of natural polymers. In living organisms the absorption of UV light can lead to photoaging of skin. UV light can be also considered as a tool for surface modification of biopolymeric materials. During UV-irradiation of biopolymers, the excited molecules can be formed in the first stage, and then the secondary processes such as chain scission, cross-linking, and oxidation take place. The above processes can alter physico-chemical properties of biopolymers [1,2].

In recent years, not only one separate biopolymer, but also the bends of two or more biopolymers have appeared to be more and more interesting for scientists and technologists [3]. Special attention has been paid on new materials based on the blends of two natural polymers containing inorganic nanoparticles [3].

The purpose of this study was to compare the results of modification of several natural polymers and their composites by UV-irradiation. Photochemical stability of single biopolymers has been studied widely [4–8]. It was found that after UV-irradiation of proteins the alteration of primary, secondary and tertiary structure occurred [9,10]. For polysaccharides the alteration of the molecular weight under UV-irradiation was observed [11,12]. It was also found that several low and high molecular weight compounds can modify the photochemical stability of natural polymers [13,14]. Moreover, low doses of UV radiation can stimulate cell activity in several biopolymer-based materials [15]. Photochemical stability of several polymer blends was studied previously [16–18]. However, the most important for several applications are photochemical modification of the surface of composite materials.

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In this study films made of biopolymers were irradiated in air at room temperature using a Philips TUV-30 mercury lamp, which emits light at a wavelength of 254 nm. Moreover, they were irradiated with KrF laser light with a wavelength of 248 nm.

Materials and Methods

Collagen was obtained in our laboratory from tail tendons of young albino rats. Briefly, tendons were excised and washed in distilled water, and blended in a Waring blender in 0.5M acetic acid, samples were then spun at 10,000 rpm in a Sorvall centrifuge and the soluble fraction decanted and lyophilised.

Silk fibroin was obtained in our laboratory from Bombyx mori. Bombyx mori silk fibroin was boiled for 1 h in an aqueous solution of 0.5% Na_2CO_3 and then rinsed thoroughly with distilled water to extract the sericin proteins. The degummed silk was dissolved in $CaCl_2/H_2O/CH_3CH_2OH$ solution (molar ratio: 1:8:2) at 80°C. Then the fibroin solution was filtered and dialyzed against distilled water for 3 days to yield a fibroin aqueous solution. The final fibroin concentration was 5%, (it was determined by weighing the remaining residue after drying).

Chitosan of low molecular weight was supplied by the company Sigma–Aldrich (Poznan, Poland). The deacetylation degree (DD,%) of chitosan was 80% and it was determinated by the conductometric titration method, taking the first derivative of the conductivity of the chitosan solution (dissolved in 0.1 M HCl) and the volume of the titration solution (0.1 M NaOH). The molecular weight of chitosan was determined by the viscometric method using the well-known Mark-Houwink equation. The intrinsic viscosity of CTS was measured with an Ubbelohde viscometer in 2% acetic acid/0.2 M sodium acetate solution.

Biopolymer films were obtained by casting the mixture of two biopolymers with different composition in solution onto a glass plate, and - after the evaporation of solvent - the film samples were dried in vacuum at room temperature. The films were irradiated in air at room temperature using a mercury lamp, Philips TUV-30, which emits light of mainly 254 nm wavelength. The intensity of radiation was 0.154 $J/(s^{2}{*}m)$. The dose of incident radiation during 1 h of exposure was 25.7 W/m^2. The intensity of the incident light was measured using an IL 1400 A Radiometer (International Light, USA).

The interactions between two biopolymers were evaluated by infrared spectroscopy using a Genesis II FTIR spectrophotometer (Mattson, USA) equipped with an ATR device (MIRacle^{TM} PIKE Technologies) with zinc selenide (ZnSe) crystal. All spectra were recorded in absorption mode at 4 cm^{-1} intervals and 64 scans. The measurements on all of the samples were repeated at different locations.

The topographic imaging was performed in air using a commercial AFM apparatus (NanoScope III Model Dimension 3100, Digital Instruments, Santa Barbara, CA) operating in contact mode. All samples were imagined using the cantilever with spring constant of 0.06 N/m and silicon nitride tip of 10 nm radius. Surface images, using the scan widths ranging from 4 to 20 μm with scan rate of 0.7 Hz, were acquired at fixed resolution (512 × 512 data points).

The contact angles of two liquids: diiodomethane (D) and glycerol (G) on the surface of biopolymeric films were measured at constant temperature of 22°C, using a goniometer equipped with the system of drop-shape analysis (DigiDrop, DGD, Fast/60, GBX, France and DSA 10 produced by Krüss, Germany). The liquid drop of 2–3 μl was placed onto the polymer surface with a microsyringe. The drop image was recorded by a video camera and digitalized. The profile of single drop was numerically solved and fitted by appropriate

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mathematical functions. Each accepted result of contact angle is the average value of maximum 10 measurements, whose precision was 0.2°. The surface free energy and its polar and dispersive components were calculated using Owen-Wendt method and the formula below [19].

$$\gamma_L * \frac{1+\cos\theta}{2} = \left(\gamma_S^d * \gamma_L^d\right)^{1/2} + (\gamma_S^p * \gamma_L^P)^{1/2} \tag{1}$$

where: $\gamma_S{}^p$ – polar component of surface free energy
$\gamma_L{}^p$ – dispersive component of surface free energy

Results and Discussion

Thin films from mixture of collagen, chitosan and silk fibroin in solution were prepared by solvent evaporation. The interactions between two biopolymers were evaluated by infrared spectroscopy. The shift of typical bands in FTIR spectra showed that there were interactions via hydrogen bonding between the components of the blends [3,5,18,20].

For the films obtained from the biopolymers mixture the surface properties, such as wettability and roughness, were measured. The contact angle for two different liquids (diiodomethane – D and glycerol - G) on the surface of biopolymeric films was measured before and after UV-irradiation. The contact angle is a measure of non-covalent forces between liquid and the first monolayer of tested material. Thus, in case of strong interactions between the studied phases, the liquid drop spreads on the solid and wets it.

Using the contact angle measurements the values of surface free energy were calculated by Owen-Wendt method. After UV irradiation the values of surface free energy increased for all the blends studied. The calculations of dispersive ($\gamma_s{}^d$) and polar ($\gamma_s{}^p$) components of surface free energy gave more detailed information about the surface properties of samples studied. Polar component of surface free energy for chitosan films is much smaller than that of silk fibroin film and collagen film. The values of polar components of surface free energy for chitosan/silk fibroin blends are similar to those for silk fibroin. UV-irradiation of chitosan, collagen, silk fibroin and their blends caused alterations of both, dispersive and polar components of surface free energy. Generally, the dispersive component of surface free energy decreased with increasing time of UV-irradiation, but one can observe several irregularities. The polar component of surface energy increased with the increasing irradiation time for all studied samples (Figure 1). The increase of polarity of samples indicates that efficient photooxidation took place on their surface. Free radicals formed during UV-irradiation may react with atmospheric oxygen leading to the formation of different oxygen-containing groups, namely of carbonyl, hydroxyl and hydroperoxide type, strongly altering $\gamma_s{}^p$. The big changes of this parameter observed for chitosan films and SF films prove that photoxidation of chitosan and SF on the surface is very efficient.

The surface properties of biopolymeric films were observed using Atomic Force Microscopy (AFM). The examples of AFM images of silk fibroin film before and after UV-irradiation are shown in Figure 2.

The surface morphology of films made of collagen, chitosan, silk fibroin and their mixtures is different. The silk fibroin top layer as well as the collagen top layer are organized in a characteristic pattern, while chitosan has a relatively smooth and flat surface and does not contain any symptom of surface roughness.

To compare surface properties of biopolymer films before and after UV-irradiation the Atomic Force Microscopy (AFM) was used and surface roughness was evaluated.

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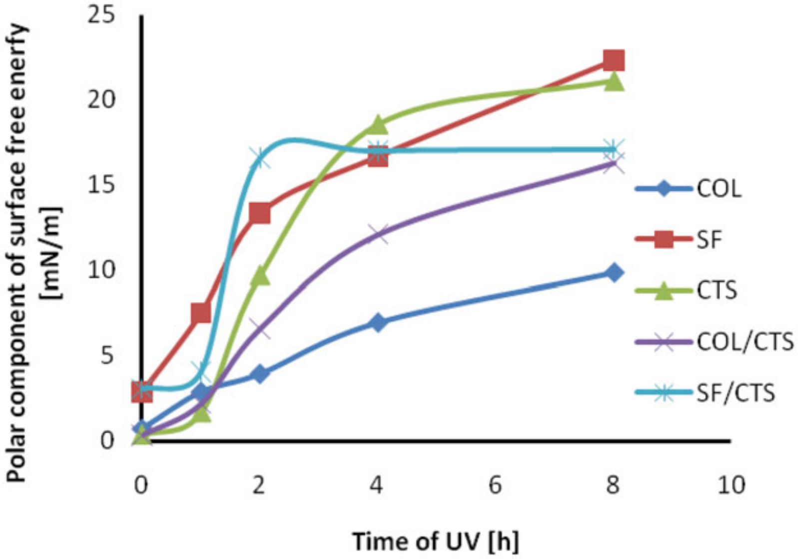
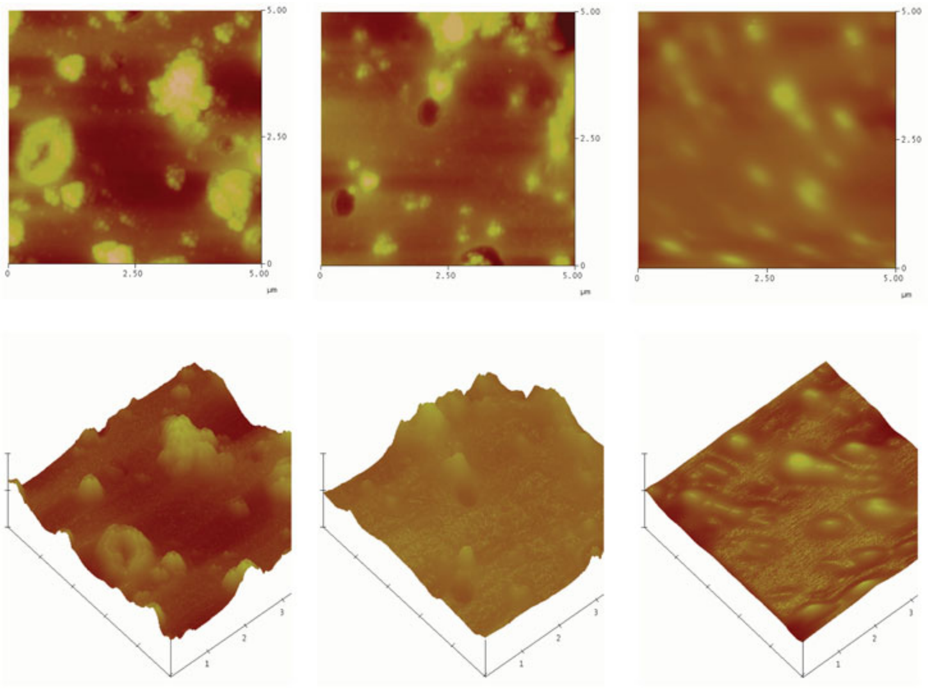


Figure 1. Polar components of surface free energy of biopolymeric films made of silk fibroin, chitosan, collagen and their blends (50/50) before and after UV-irradiation (COL-collagen, SF-silk fibroin, CTS-chitosan).



0 h UV 2 h UV 8 h UV

Figure 2. AFM image of the surface of the film made of silk fibroin before and after 2 and 8 hours of irradiation.

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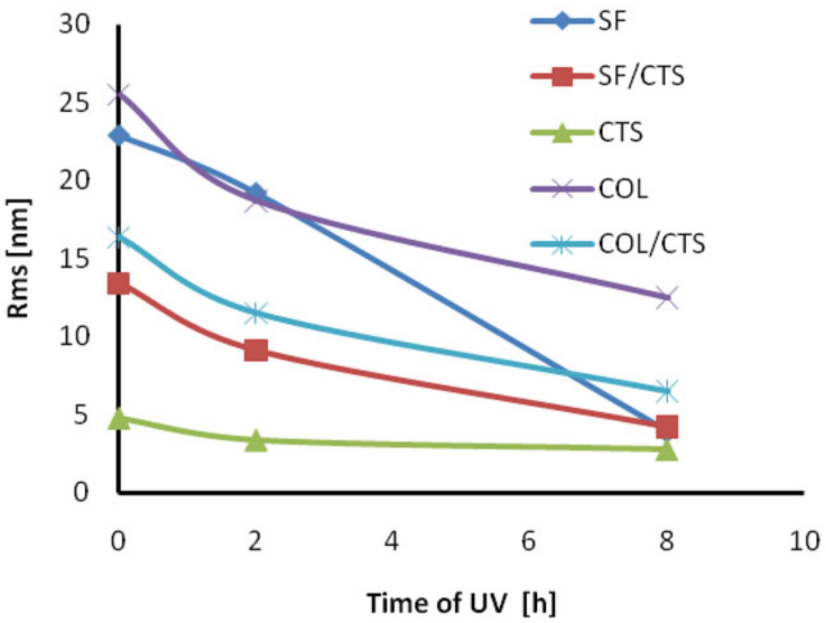

Figure 3. Surface roughness of biopolymeric films made of silk fibroin, chitosan, collagen and their blends (50/50) before and after UV-irradiation (COL-collagen, SF-silk fibroin, CTS-chitosan).

Small topographical changes in AFM images of biopolymer films were observed to take place during UV-irradiation. AFM images show differences in surface properties of collagen films, chitosan films, silk fibroin films and films made of the blend of the above biopolymers before and after UV-irradiation. The roughness of films made of silk fibroin and those made of collagen is rather big (about five times bigger than that of chitosan film). When the blend contains 50% of chitosan and 50% of silk fibroin the roughness is smaller than for silk fibroin but much bigger than for chitosan film. Such roughness can be a result of molecular architecture of silk fibroin macromolecules. When the blend contains 50% of collagen and 50% of chitosan the roughness is smaller than for collagen film but much bigger than for chitosan film. Again, such big roughness for collagen film can be a result of molecular architecture of collagen macromolecules. Structural proteins such as silk fibroin and collagen possess hierarchical structure and thus during solvent evaporation the hierarchical structure may lead to big roughness of the surface. The atomic force microscope is often used to obtain a reasonable measure of surface roughness on the nanometer scale. Typically, AFM users rely on root-mean-square (*Rms*) roughness. The *Rms* surface roughness of collagen, chitosan, silk fibroin and the blended films has been shown in Figure 3. *Rms* value for chitosan/silk fibroin film is much bigger than for pure chitosan film, but it is smaller than for silk fibroin film. *Rms* value for collagen/chitosan film is much bigger than for pure chitosan film, but it is smaller than for collagen film The surface of chitosan, silk fibroin, collagen and chitosan/silk fibroin blended films has been altered by UV-irradiation. UV-irradiation caused the decrease of surface roughness of chitosan/collagen films, as well as silk fibroin film and film made of the blend of chitosan and silk fibroin (50/50).

Previously we found, that UV-irradiation of silk fibroin in solution led to the new photoproducts formed during UV exposure as well as led to partial degradation of silk

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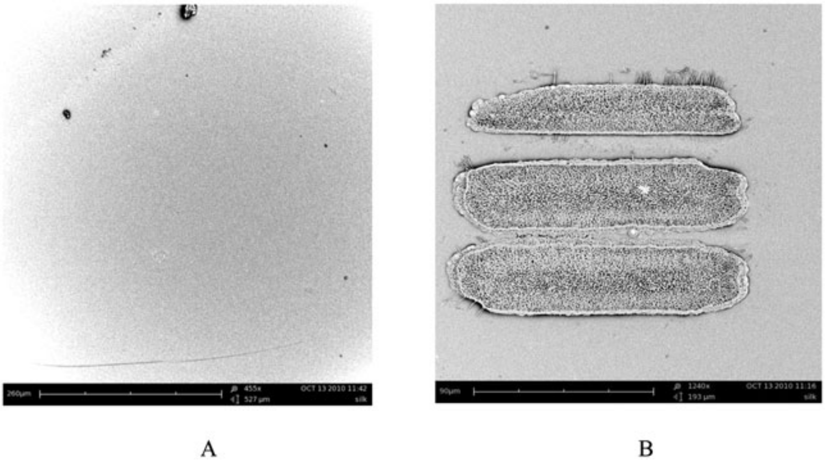

Figure 4. SEM images of the surface of silk fibroin film (A) and the surface of silk fibroin film treated with nanosecond KrF laser beam with one pulse of fluence 3 J/cm^2 and the wavelength 248 nm (B).

fibroin [5,20]. The presence of silk fibroin in chitosan matrix can enhance photochemical processes in the blend as silk fibroin contains aromatic amino acids capable to absorb UV light. It was also found previously that UV-irradiation of collagen in solution led to the new photoproducts formed during UV exposure as well as led to partial degradation of collagen [9,10,13].

In this study films made of biopolymers were irradiated by UV light from incoherent lamp and they were irradiated with KrF laser light with a wavelength of 248 nm. However, it has to be mentioned that the energy of both UV sources was different. UV light emitted by lasers is much more destructive than energy emitted by incoherent lamp.

The contact angle measurements for KrF laser irradiated biopolymer films are strongly influenced by the roughness. Surface roughness is source of large contact angle hysteresis, which becomes the dominant effect and completely masks the influence of chemical composition. Therefore a more suited characterization of the laser treated surface is SEM. The silk fibroin top layer seen by SEM before laser irradiation has a relatively smooth and flat surface. The SEM images of silk fibroin films before after laser treatment are shown in Figure 4. The material expansion into a new foam structure can easily be seen in the SEM images. For biopolymer films the treated surface in general rises approximately by several nm for the fluence 3 J/cm^2.

The mechanism of micro-foam formation on several biopolymer films' surface has been discussed in previous paper regarding laser ablation [21,22].

Conclusions

After UV-irradiation of biopolymeric films made of the blends of silk fibroin, collagen and chitosan with the wavelength 254 nm several alterations were observed. The contact angle measurements and values of surface free energy showed that the wettability of biopolymeric films was changed by UV- irradiation.

KrF laser treatment caused more significant damage of the surface of biopolymeric films made of the blends of silk fibroin, collagen and chitosan in comparison to UV-irradiation emitted by incoherent lamp. On several biopolymeric films after laser treatment the formation of the micro-foam was observed.

Overall, the processes induced by UV light in biopolymers and biopolymer composites can be used for surface modification of several materials for potential biomedical applications.

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References

[1] Fischbach, C., Tessmar, J., Lucke, A., Schnell. E., Schmeer, G., & Blunk T. (2001). *Surf. Sci.*, *491*, 333.

[2] Kaczmarek, H., Kowalonek, J., Szalla, A., & Sionkowska A. (2002). *Surf. Sci.*, *507 510*, 883.

[3] Sionkowska, A. (2011). *Prog. Polym. Sci.*, *36*, 1254.

[4] Smith, G. J. (1995). *J. Photochem. Photobiol. B.*, *27*, 187–198.

[5] Sionkowska, A., & Planecka, A. (2011). *Polym. Deg. Stab.*, *96*, 523.

[6] Wiśniewski, M., Sionkowska, A., Kaczmarek, H., Lazare, S., Tokarev, V., & Belin, C. (2007). *J. Photochem. Photobiol. A.*, *188*, 192.

[7] Sionkowska, A. (2006) *J. Photochem. Photobiol. B.*, *84*, 38.

[8] Sionkowska, A., &. Wess, T. J. (2004). *Inter. J. Biol. Macromol.*, *34*, 9.

[9] Miles, C. A., Sionkowska, A., Hulin, S. L., Sims, T. J., Avery, N. C., & Bailey, A. J. (2000). *J. Biol. Chem.*, *275*(42), 33014.

[10] Sionkowska, A. (2005) *J. Photochem. Photobiol. B.*, *80*, 87.

[11] Wang, S. M., Huang, Q. Z., & Wang, Q. S. (2005). *Carbohyd. Res.*, *340*, 1143.

[12] Wasikiewicz, J. M., Yoshi, F., Nagasawa, N., Wach, R. A., & Mitumo, H. (2005). *Rad. Chem. Phys.*, *73*, 287.

[13] Sionkowska, A., Kamińska, A., Miles, C. A., & Bailey, A. J. (2001). *Polimery*, *6*, 379.

[14] Kamińska, A., & Sionkowska, A. (1996). *J. Photochem.Photobiol. A.*, *96*, 123.

[15] Rajan, N., Couet, F., Pennock, W., Lagueux, J., Sionkowska, A., & Mantovani, D. (2008). *Biotech. Prog.*, *24*(4), 884.

[16] Sionkowska, A. (2006). *J. Photochem. Photobiol. A.*, *177*, 61.

[17] Sionkowska A. (2006). *Polym. Deg. Stab.*, *91*, 305.

[18] Sionkowska, A. (2013). *In: Polymeric Biomaterials*, Third Edition. S. Dumitriu, S., & V. Popa, V. (Eds.), Chapter 11. Taylor & Francis Inc CRC Press: Boca Raton, 309.

[19] Adamson, A.W. (1990). *Physical Chemistry of Surfaces, 5th ed.*, Wiley-Interscience, New York.

[20] Sionkowska, A., & Planecka, A. (2013). *J. Mol. Liq.*, *178*, 5.

[21] Lazare, S., Tokarev, V., Sionkowska, A., & Wisniewski, M. (2005). *Appl. Phys. A: Mater. Sci. Proces. 81*, 465.

[22] Lazare, S., Sionkowska, A., Zaborowicz, M., Planecka, A., Lopez, J., Dijoux, M., Loumena, C., & Hamande, M.C. (2012). *Appl. Phys. A: Mater. Sci. Proces.*, *106*, 67.

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