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# Effect of UV-light on the uniaxial tensile properties and structure of uncoated and $TiO_2$ coated *Bombyx mori* silk fibers

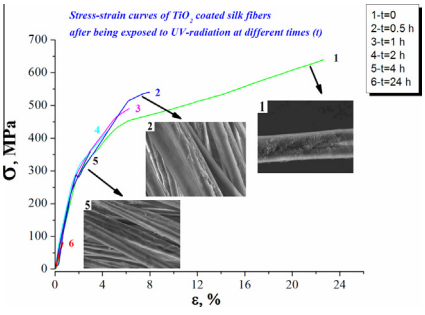
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## HIGHLIGHTS

- $E_y$ value of $TiO_2$ coated silk fibers became higher than uncoated silk fibers after UV-irradiation.
- A plastic deformation region was not observed for the silk fibers after UV-irradiation for $t > 1$ h.
- Less photodegradable β-sheet microcrystals by the effect of titanium dioxide coating.
- UV-irradiation decreased dramatically breaking extension value of silk fibers.
- After 1 day UV-irradiation, Young's modulus of $TiO_2$ coated silk fibers became ~13 GPa.

## GRAPHICAL ABSTRACT




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## ABSTRACT

The effect of UV-light on the uniaxial tensile properties and the structure of uncoated and $TiO_2$ coated silk fibers in the bave form by using sol-gel method was investigated with tensile testing and FT-IR/ATR spectroscopy methods after the silk filaments were exposed to UV-light with high intensity of 760 W/m$^2$ for different times from 0.5 h to 1 day. It was clearly observed that $TiO_2$ coating considerably increased the Young's modulus of the uncoated silk single filament by around 17% before the UV-irradiation. The yield point and the post yield region disappeared on the stress-strain curves of both uncoated and $TiO_2$ coated silk filaments after UV-irradiation time higher than 1 h. Except for the Young's modulus, most of the tensile characteristics of both uncoated and $TiO_2$ coated silk filaments decreased remarkably with increasing UV-irradiation time, e.g., after 1 h irradiation, although the Young's modulus slightly changed and ultimate tensile strength decreased by only around 18% and 23%, for the uncoated and $TiO_2$ coated silk filaments, respectively; breaking extension decreased dramatically by 67% and 72%, respectively, for uncoated and $TiO_2$ coated silk filaments. Only the Young's modulus of $TiO_2$ coated silk filaments which can be considered as a more stable tensile characteristic became significantly higher than that of the uncoated silk filaments with increasing UV-irradiation time. After 1 day irradiation, even though the uncoated silk filaments could not be tested and completely lost of their fiber properties, the $TiO_2$ coated silk filaments showed a stress-strain curve in initial elastic region with Young's modulus of ~13 GPa which indicates considerable protective effect of $TiO_2$ on the silk fiber structure, especially on the β-sheet microcrystals against UV-radiation. The FT-IR/ATR spectral results showed that significant photodegradation took place in not only crystalline but also amorphous regions which were deduced from the decrease in the absorbance ratios of the bands assigned to $CH_3$ rocking, $C_\alpha$–$C_\beta$, $C_\alpha$–C stretching vibrations in β-sheet crystalline regions as well as the Amide I, II, and III bands for both crystalline and amorphous regions. Even though the ratio of crystalline to amorphous regions in uncoated silk filaments decreased

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significantly, the ratio in $TiO_2$ coated silk filaments became almost constant with increasing UV-irradiation time which may indicate more stable β-sheet microcrystals against photodegradation.

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## Introduction

*Bombyx mori* (*B. mori*) silk because of having not only unique mechanical properties such as high tensile strength and stiffness [1] but also other properties such as biocompatibility and biodegradability [1–5] has a great potential applications and attracts great attentions as a research subject in different fields ranging from textile material to composite science, biotechnology [3–5], medicine, and tissue regeneration [2] in different forms such as silk sutures and silk fibroin biomaterials as well as its fiber and yarn form. *B. mori* silk fiber is composed of two protein components, that is, silk fibroin cores known as monofilaments or brins being the main part of silk fiber and amorphous silk sericin coating and surrounding them [6,7]. Microstructure of silk fibroin consists of highly ordered crystalline regions known as β-sheet micro crystals (crystalline phase) in which polypeptide chains having the repeating amino acid sequence of [-Gly-Ala-Gly-Ala-Gly-Ser-]*n* with the ratio of 3:2:1 are well ordered with anti-parallel arrangement in β-pleated sheets and of amorphous flexible chains containing also the larger amino acid residues such as tyrosine and aspartic acid, and valine in addition to the three residues mentioned above [8–12]. The β-sheet microcrystals are stabilized by intra-sheet hydrogen bonds between carbonyl oxygens and amide hydrogens of adjacent polypeptide chains in the same β-pleated sheet and by inter-sheet Van der Waals interactions occurring between hydrophobic side chains [10].

In order to enhance the properties of silk fibers or silk-based biomaterials such as regenerated silk fibroin films, from inorganic particles, titanium dioxide ($TiO_2$) nanoparticles being cheap, non-toxic, and photostable have been extensively used [3,4,13,14]. Due to photocatalytic activity of nano-$TiO_2$ particles, textile fabrics treated with nano-$TiO_2$ particles could provide effective protection against bacteria and the discoloration of stains [15,16]. As an inorganic UV-blocker, nano-sized titanium dioxide was used for cotton fabrics to increase of the effectiveness of blocking UV radiation [17,18]. It was also shown by Feng et al. [4] that with increasing amount of $TiO_2$ in silk fibroin/nano-$TiO_2$ composite films, the mechanical properties of the films were improved. In our previous study, we also showed that $TiO_2$ coating by sol-gel process improved especially Young's modulus of silk yarns even though it decreased breaking extension little [19].

Although the mechanical and some other properties of silk composites films based on silk fibroin and $TiO_2$ and of $TiO_2$ coated silk yarns were investigated [4,13,14,19–22], how the uniaxial tensile properties of $TiO_2$ coated silk filaments by sol-gel method change after the effect of UV-irradiation with high intensity have not been investigated at all according to our knowledge.

Thus, in this study it was aimed to investigate the changes in the tensile properties of $TiO_2$ coated silk filaments after they were exposed to UV-irradiation of high intensity for different times from 0.5 h to 24 h and compare with those of uncoated silk filaments by using tensile testing method. SEM (scanning electron microscope) method was also applied to the irradiated silk filaments in order to see the changes in their surface morphology. Moreover, Fourier transform infrared [FT-IR]/attenuated total reflectance [ATR] method was used to understand the structural changes induced by the UV photo-degradation which are responsible for the changes in the tensile properties of both uncoated and $TiO_2$ coated silk filaments after UV-irradiation.

## Materials and methods

### *Materials and UV-irradiation process*

#### *Uncoated B. mori silk fibers*

Silkworm (*B. mori*) silk cocoons purchased from Koza Han in Bursa, Turkey were opened and degummed with the method described earlier [23]. Because the degummed silk fibers were obtained in the form of bave consisting of the two constituent brins of the cocoon fiber which could not be separated. Then, the degummed silk fibers were dried at room conditions ($T$ = 25 °C; RH = 60%) for at least 24 h before performing any experiment.

#### *Preparation of $TiO_2$ sol and $TiO_2$ coated silk fibers*

Before coating the silk fibers with titanium dioxide, titanium dioxide sol was prepared following the method described earlier [19,24] in two steps using titanium (IV) butoxide ($Ti[O(CH_2)_3CH_3]_4$, TIVBT) [97% Sigma-Aldrich, USA]. In the first step, TIVBT, acetic acid ($CH_3COOH$) [100% Merck, Germany], and ethanol (EtOH) [99.8% Riedel-de Haën, Germany] were mixed at room temperature for 30 min with the molar ratios TIVBT:EtOH:$CH_3COOH$ = 1:113.45:1.4. In the second step, ethanol was added to the solution so that the molar ratios became TIVBT:EtOH:$CH_3COOH$ = 1:189.1:1.4. After this, the solution was stirred for 1 h more at room temperature and then the titanium dioxide sols became homogeneous and transparent.

In order to coat the silk baves with $TiO_2$ each silk bave having the length of approximately 15–20 cm was kept in the $TiO_2$ sol for 3 min and after being fixed with clamps from its tips, it was held parallel to the table at a distinct height to dry at room conditions for 1 h.

#### *Ultraviolet (UV) irradiation of the silk samples*

Both uncoated and $TiO_2$ coated silk fibers (baves) were irradiated in a metal chamber using an Osram ULTRAMED-1000 W lamp which emits high radiation intensity in the ultraviolet range from 280 to 400 nm. The UV lamp emits light at wavelength of 280-315 nm (UVB) and 315-400 nm (UVA). The intensity of radiation was 760±15 W/m$^2$ which was measured using a Radiometer. For tensile tests, before UV irradiation, each bave sample was stuck in a rectangular paper frame so that the gauge length became 5 cm. Then, the prepared samples were placed very close and parallel to each other and irradiated under the UV lamp for different times. For spectroscopic and SEM measurements, silk bave samples with approximately 10 cm in length were placed very close and parallel to each other and then were irradiated under the UV lamp.

### *Methods*

#### *Tensile testing method and measurements*

Uniaxial tensile tests of *B. mori* silk filament with 50 mm gauge length were made on a Lloyd tensile testing machine LF *Plus*, (AMETEK Lloyd Inst. Ltd., UK) operating at a cross-head speed of 50 mm/min at room conditions. In order to examine the influence of UV-radiation, both uncoated and $TiO_2$ coated silk fibers were irradiated for different times from 0.5 to 24 h and then kept at room conditions for 1 day prior to the tensile test. 15 samples were tested for each UV-irradiation treatment and the results concerning the tensile properties were averaged.

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*Scanning electron microscopy (SEM)*

Scanning electron microscope images of the uncoated and $TiO_2$ coated silk fibers and of irradiated $TiO_2$ coated silk fibers were obtained by using SEM microscope (Model QUANTA 650 FEG) with an applied high voltage of 20.0 kV and different magnifications ranging from 1000 to 2000.

*FT-IR/ATR spectroscopy*

FT-IR/ATR experiments were carried out on a Perkin–Elmer Fourier transform spectrometer "Spectrum One" (Perkin Elmer, Inc. USA). The infrared spectra of the samples before and after UV-irradiation at different times were recorded on the spectrometer equipped with ATR crystal cell in the 550–4000 $cm^{-1}$ spectral range at a resolution of 4 $cm^{-1}$ with 4 scans for each measurement. Approximately 40 individual silk baves which were placed very close and parallel to each other were attached to small rectangular frames and fixed by using a strong glue (Pattex Super Glue, Germany). Also, the FT-IR/ATR spectrum of $TiO_2$ powder with sufficient amount (~1 mg) after being clamped against the vertical face of the ATR crystal was recorded.

## Results and discussion

*Results*

*SEM results of the silk filaments after the effect of UV-irradiation*

The SEM images of the uncoated and $TiO_2$ coated silk filaments before the effect of UV-irradiation are shown in Fig. 1. It is apparently seen that although the surfaces of uncoated silk filaments are clear and smooth, after coating process, the surface of the silk filaments were covered with a thin $TiO_2$ layer and due to the excessive amount of $TiO_2$ particles, the accumulation of these $TiO_2$ particles on some parts of the surface of silk filaments was also observed.

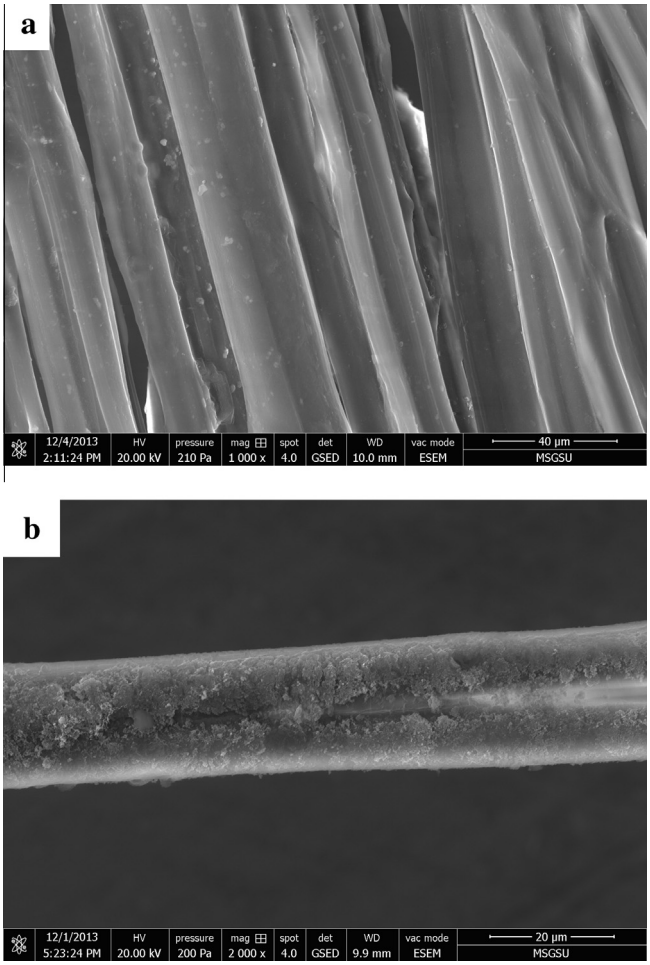



**Fig. 1.** SEM microphotographs of (a) uncoated and (b) $TiO_2$ coated silk filaments.

Here, we think that $TiO_2$ network were formed on the surface of silk filaments via the interaction of the $TiO_2$ sol with OH groups of especially, serine in the crystalline regions and tyrosine amino acids in the amorphous regions by means of hydrolysis and the condensation reactions of the sol-gel method as shown in our previous study done on *B. mori* silk yarns [19]. On the other hand, the changes on the surfaces of the $TiO_2$ coated silk filaments after the filaments were irradiated by UV light for different times were shown in Fig. 2.

It is clearly seen that some micro and macro cracks were formed on the thin $TiO_2$ layer and on the accumulated parts of $TiO_2$ coating on the surfaces of the silk filaments. With increasing time of UV-irradiation, the deformation on the $TiO_2$ layer increased, that is, the amounts of the cracks increased. However, although the intensified UV-irradiation was applied to the fibers, great photo degradation or destruction resulting in the rupture or failure of the silk fibers was not seen on the surface or the bulk of the silk filaments (Fig. 2).

*Uniaxial tensile properties of the uncoated and $TiO_2$ coated silk filaments exposed to UV-radiation*

The stress-strain curves of both uncoated and $TiO_2$ coated silk fibers (bave) exposed to UV-radiation at different times are shown respectively, in Figs. 3 and 4. The influence of UV-irradiation in the uniaxial tensile properties of the uncoated and $TiO_2$ coated silk fibers is tabulated in Tables 1 and 2. It is known that a typical stress-strain curve of silk fiber consists of an initial elastic deformation region up to around 2% strain and following a yield point at around 4-5% strain, a plastic deformation region.

In the plastic deformation region great structural changes and degradation processes occur, e.g., inter-chain hydrogen bonds between polypeptide chains in amorphous regions and the chains interacting with crystalline regions may be broken at around or past the yield point, therefore, such chains may be extended and slide and past each others and crystalline regions, which result in plastic deformation [12]. For further stretching, these chains can be more extended and oriented along the stretching direction as well as the orientation of β-sheet crystalline units. During stretching and orientation process, changes in the inter-chain and intra-sheet hydrogen bonds can be seen and also breakage in some chemical bonds especially in amorphous chains at high strain values can be expected.

Comparison of the stress-strain curves of the uncoated and $TiO_2$ coated silk fibers shows that the shape of the stress-strain curve of $TiO_2$ coated silk fibers is very similar to that of uncoated silk fibers consistent with the results shown for *B. mori* silk yarns in the study [19]. Even though $TiO_2$ coated silk filament obtained relatively higher tensile properties such as tensile strength, breaking extension, it obtained considerably higher Young's modulus and work of rupture than those of uncoated silk filament, that is, Young's modulus increased by around 17% and work of rupture were increased by around 11% (Tables 1 and 2). These higher tensile properties indicate that $TiO_2$ coating significantly contributed to Young's modulus and led to relatively stronger and stiffer silk filament.

After both uncoated and $TiO_2$ coated silk filaments underwent UV-irradiation, with increasing time of irradiation, some significant changes were seen on the stress-strain curves of both uncoated and $TiO_2$ coated silk filaments. The extensibility decreased dramatically, even after the filaments were irradiated under UV-light for the time higher than 1 h, a yield point could not be determined; hence, the yield point and the plastic deformation region disappeared. This is quite important result that

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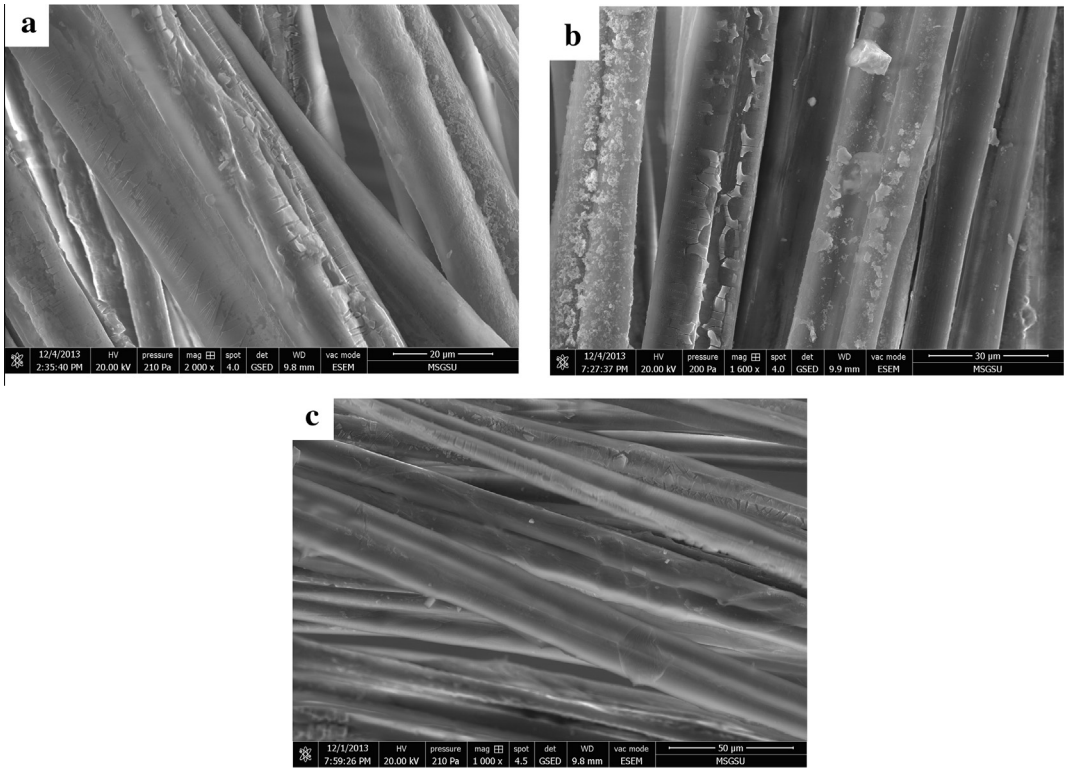



**Fig. 2.** SEM images of $TiO_2$ coated silk filaments after being exposed to UV-radiation at different times: (a) 0.5 h, (b) 2 h, (c) 4 h.

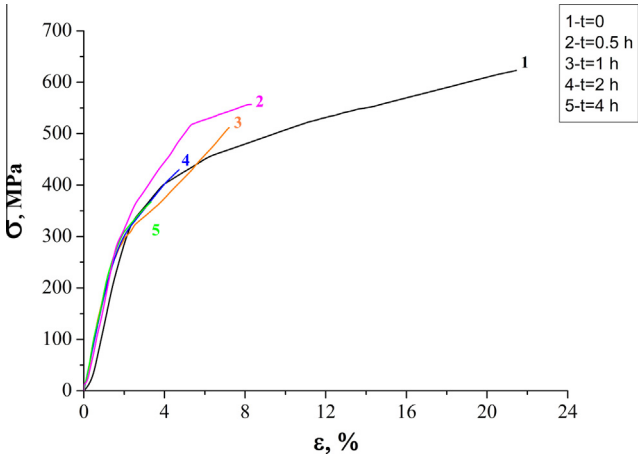



**Fig. 3.** Representative stress-strain curves of uncoated silk filaments after being exposed to UV-radiation at different times ($t$).

supports the idea that the amorphous flexible chains were more deformed and broken with the effect of photodegradation and photodestruction than those in β-crystalline regions. Considering the effect of UV-radiation on the tensile properties, it is seen that in particular the irradiated silk filaments greatly lost tensile strength, extensibility or breaking extension, and work of rupture values. For instance, even after the silk filaments were irradiated under UV light for 1 h, although ultimate tensile strength decreased similarly by only around 18% and 23%, for the uncoated and $TiO_2$ coated silk filaments, respectively, breaking extension decreased greatly by 67% and 72%, and work of rupture decreased by 87% and 90% for uncoated and $TiO_2$ coated silk filaments, respectively. In contrast to the dramatic changes above, the Young's modulus of both the uncoated and $TiO_2$ coated silk filaments did not change much. It should be noticed that Young's modulus of $TiO_2$ coated silk filaments remained significantly higher than that of the uncoated silk filaments as seen in Tables 1 and 2, e.g., after the uncoated and $TiO_2$ coated silk filaments were irradiated by UV-light for 4 h, the Young's modulus of the $TiO_2$ coated silk filaments became ~11% higher than that of the uncoated silk filaments. Moreover, after both uncoated and $TiO_2$ coated silk filaments were irradiated for 24 h under UV light, although the uncoated silk filament could not be tested mechanically at all by using the tensile tester because of being very weak and brittle and loosing all of its tensile properties dramatically, the $TiO_2$ coated silk filament obtained a stress-strain curve in the initial elastic region and showed at least a significant resistance and low tensile properties in the initial elastic deformation region thanks to the protective $TiO_2$ coating

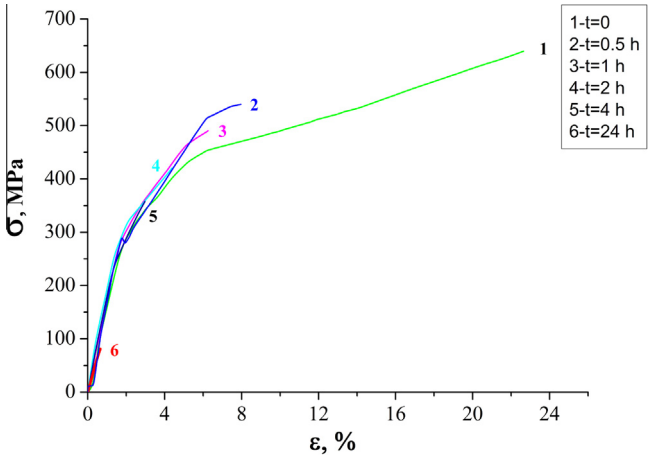



**Fig. 4.** Representative stress-strain curves of $TiO_2$ coated silk fibers after being exposed to UV radiation at different times ($t$).

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**Table 1**
Uniaxial tensile properties of the uncoated *B. mori* silk filaments after being exposed to UV-light at different times ($t$).

| $t$ (h) | $E_y$ (GPa) | $\varepsilon_y$ (%) | $\sigma_y$ (MPa) | $\varepsilon_b$ (%) | $\sigma_u$ (MPa) | $U$ (mJ) |
|---|---|---|---|---|---|---|
| 0 | 17.9 | 4.7 | 465 | 21.5 | 623 | 0.736 |
| 0.5 | 17.8 | 4.5 | 494 | 8.3 | 557 | 0.152 |
| 1 | 17.5 | 3.5 | 435 | 7.2 | 512 | 0.093 |
| 2 | 18.2 | – | – | 4.7 | 430 | 0.065 |
| 4 | 17.7 | – | – | 3.3 | 367 | 0.029 |
| 24 | – | – | – | – | – | – |

against photodestruction. Especially, the observation of Young's modulus being at around 13 GPa indicates the photodegradation and photodestruction occurred less in strong β-sheet microcrystals which contributed greatly to the stiffness and Young's modulus of the $TiO_2$ coated silk filament than in amorphous regions. In other words, it can be thought that more macromolecular polypeptide chains in amorphous regions were damaged and broken which resulted in the dramatic decrease in the breaking extension and tensile strength.

*FT-IR/ATR results of the uncoated and $TiO_2$ coated silk filaments exposed to UV-radiation*

The FT-IR/ATR spectra of $TiO_2$ powder, uncoated and $TiO_2$ coated silk filaments are shown in Fig. 5. It is known that the peak at 550 $cm^{-1}$ was attributed to stretching vibration of Ti-O in the studies [25–27], hence, in the spectra of pure $TiO_2$ powder as seen in Fig. 5, the peak observed in the 570-670 $cm^{-1}$ region with a maximum at 650 $cm^{-1}$ is likely due to the vibration of Ti-O bonds. The peak at 801 $cm^{-1}$ is assigned to –TiO–Ti– stretching [28]. The bands observed at 1028, 1415, and 1527 $cm^{-1}$ were attributed to stretching and vibrations of the Ti–O–Ti group [29]. The peaks at 2853 and 2924 $cm^{-1}$ were attributed to the symmetrical and antisymmetrical C-H stretching vibration of $CH_2$ [30,31], respectively. A broad band centered at about 3180 $cm^{-1}$ is associated with the hydrogen-bonded OH stretching vibrations [14].

Fundamental IR bands observed in the spectrum of silk fibers and silk fibroin are tabulated in Table 3. Here, the band at around 700 $cm^{-1}$ which was observed at 690 $cm^{-1}$ in the spectra of uncoated and $TiO_2$ coated silk filaments is assigned to Amide V band [30,32]. The weak bands at 976 and 1000 $cm^{-1}$ being not separated clearly but estimated under the peak at 1068 $cm^{-1}$ corresponding to $C_\alpha$-C stretching vibration of peptides in β-sheet conformation [33–35] are assigned respectively to $CH_3$ rock and $C_\alpha$-$C_\beta$ stretch, and $CH_3$ rock in AlaGly repeating pattern in β-sheet conformation [33,36]. Amide III mode for random-coil conformation and β-sheet conformation were observed at 1235 and 1256 $cm^{-1}$ [33,34,37]. The band observed at 1441 $cm^{-1}$ is assigned to $\delta CH_2$ scissoring [31,33,38,39].

The other two bands observed at 1516 and 1558 are attributed to the Amide II band for random-coil-conformation [33,40] and β-sheet conformation [34], respectively. Amie I band which is the C=O stretching vibration with minor contributions from the out-of-phase C–N stretching vibration and the C–N–H in plane bending

**Table 2**
Uniaxial tensile properties of the $TiO_2$ coated *B. mori* silk filaments after being exposed to UV-light at different times ($t$).

| $t$ (h) | $E_y$ (GPa) | $\varepsilon_y$ (%) | $\sigma_y$ (MPa) | $\varepsilon_b$ (%) | $\sigma_u$ (MPa) | $U$ (mJ) |
|---|---|---|---|---|---|---|
| 0 | 20.9 | 4.1 | 406 | 22.7 | 640 | 0.82 |
| 0.5 | 20.8 | 4.4 | 469 | 8.0 | 540 | 0.119 |
| 1 | 20.1 | 3.3 | 395 | 6.3 | 490 | 0.083 |
| 2 | 19.8 | – | – | 4.4 | 421 | 0.061 |
| 4 | 19.6 | – | – | 3.0 | 358 | 0.026 |
| 24 | 12.6 | – | – | 0.7 | 81 | 0.0014 |

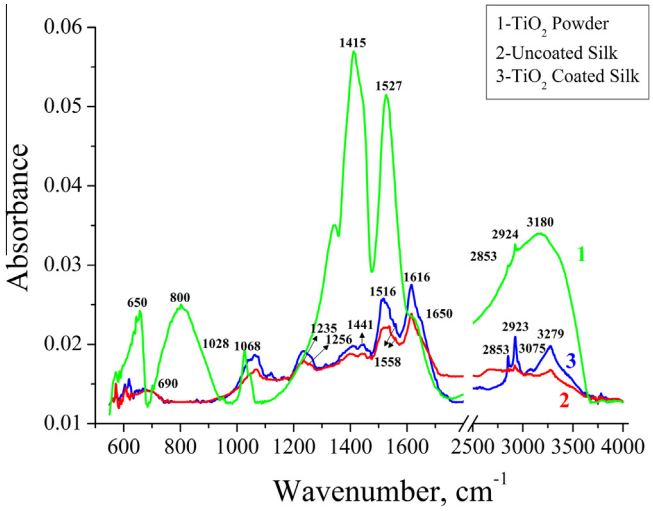



**Fig. 5.** FT-IR/ATR spectra of $TiO_2$ powder, uncoated and $TiO_2$ coated silk fibers.

**Table 3**
Peak assignments for FT-IR spectrum of silk fibers and silk fibroin.

| Wave number ($cm^{-1}$) | Assignment | Molecular group | Conformation | Refs. |
|---|---|---|---|---|
| ~700 | N–H bending | Amide V | | [30,32] |
| 976 | $CH_3$ rock, $C_\alpha$–$C_\beta$ stretch | $(AlaGly)_n$ | β-Sheet | [33,36] |
| 1000 | $CH_3$ rock | $(AlaGly)_n$ | β-Sheet | [33,36] |
| 1068 | $C_\alpha$–C stretch | Peptide | β-Sheet | [33–35] |
| ~1235 | NH bend and CN stretch and the CC stretch | Amide III | Random-coil, helical conformation | [33,34,37] |
| ~1256 | | Amide III | β-Sheet | [33,34,37] |
| 1445 | $CH_2$ scissoring | $-CH_2$ $-CH_3$ in plane | Backbone conformation | [31,33,38,39] |
| 1516 | NH out of phase with smaller C–N stretch | Amide II | Random-coil | [33,40] |
| 1558 | | Amide II | β-Sheet | [34] |
| 1616 | CO stretch | Amide I | β-Sheet | [31,33,34] |
| 1645 | | Amide I | Unordered, α-helix/random coil structure | [34,35,41] |
| 2853 | Symmetrical CH stretch | $CH_2$ | | [30,31,33] |
| 2923 | Antisymmetrical CH stretch | $CH_2$ | | [30,31,33] |
| 3075 | NH stretch | Amide B | | [33] |
| 3275 | NH stretch | Amide A | | [31,33,39] |

was observed at 1616 $cm^{-1}$ and 1645 $cm^{-1}$ for β-sheet conformation in β-sheet crystalline structures [31,33,34] and for unordered conformation [34,35,41], respectively. In the Amide A spectral region (3600-2800 $cm^{-1}$), two peaks at 2853 and 2923 $cm^{-1}$ are due to C–H stretching in the peptide chains and assigned to the symmetrical and antisymmetrical C–H stretching of $CH_2$ [30,31,33], respectively. Another peak observed at 3075 $cm^{-1}$ was attributed to N–H stretch of Amide B mode [33]. Besides, a broad peak attributed to the NH stretching of peptide groups, namely, Amide A band was observed at 3279 $cm^{-1}$ [31,33,39].

From the comparison of the FT-IR/ATR spectra of uncoated and $TiO_2$ coated silk filaments (Fig. 5), it is seen that the most of the characteristic bands of $TiO_2$ are not clearly seen on the $TiO_2$ coated silk fibers, because it is probably due to very low content of $TiO_2$ on the $TiO_2$ coated silk fibers which is compatible with our previous FT-IR spectral results obtained on $TiO_2$ coated silk yarns [19].

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However, the absorbance intensities of the bands assigned to CH stretching mode of $CH_2$ groups observed at around 2853 and 2923 $cm^{-1}$ are considerably higher than those in uncoated silk fibers, because there exist some residual $CH_2$ and $CH_3$ groups from $TiO_2$ sol during the formation of $TiO_2$ network on the silk fibers as discussed in earlier study [19].

Figs. 6 and 7 illustrate the FT-IR/ATR spectra of the uncoated and $TiO_2$ coated silk filaments after UV irradiation at different times. For the analysis of the absorbance of the bands, the absorbance ratios of the bands were calculated by using an internal standard band called $CH_2$ scissoring mode at around 1441 $cm^{-1}$ [31].

From the comparison of the absorbance ratios tabulated in Tables 4 and 5, it is generally seen that UV irradiation caused the prominent deformation and degradation in not only side groups of (AlaGly) repeating units such as $CH_3$ residues, but also some chemical bonds in the peptide chains such as $C_\alpha$-C bonds in both uncoated and $TiO_2$ coated silk fiber. It is seen that the absorbance ratios for the vibrations at 976 and 1000 $cm^{-1}$ assigned to the repeating AlaGly sequences with β-sheet conformation in silk fibroin chain decreased considerably with increasing time of UV irradiation in uncoated silk fibers. This shows that some $CH_3$ residues were greatly deformed or might be broken in polypeptide chains of β-sheet conformations in crystalline regions. In case of $TiO_2$ coated silk fibers, the relative decrease in the intensity of these bands became higher than that of uncoated silk fibers with increasing time of UV-irradiation which may indicate more deformed or degraded $CH_3$ groups in β-sheet microcrystals in $TiO_2$ coated silk fibers than those in the uncoated silk fibers.

Moreover, obvious decrease in the intensity of $C_\alpha$-C stretch in polypeptide chains at 1068 $cm^{-1}$ related to antiparallel β-sheet conformation [35] in both uncoated and $TiO_2$ coated silk fibers indicates that some $C_\alpha$-C bonds in main polypeptide chains mostly in crystalline regions were greatly deformed or broken and this decrement increased with increasing time of UV-irradiation. The FT-IR results also revealed that the absorbance values of Amide I, II, and III bands emerging from β-sheet crystalline regions and random coil conformations in amorphous regions show generally a slightly decreasing tendency in both uncoated and $TiO_2$ coated silk fibers. This effect is probably due to the weakening and/or breaking of peptide bonds in polypeptide chains as shown in the study done on the silk fibroin films [39].

In order to determine the changes in the ratio of crystalline to amorphous regions in silk fiber, the ratio of the intensity of Amide I band at 1616 $cm^{-1}$ which arises in the crystalline β-sheet structures and of Amide I band at around 1650 $cm^{-1}$ belonging to α-helices and random coils was calculated for each time of UV-irradiation. It is seen that with increasing time of UV-irradiation the crystalline ratio shows a slight decrease in the uncoated silk fibers consistent with the results concerning decrease in the crystallinity degree of silk fabrics with increasing time of UV-irradiation [33]. However, the ratio of crystalline to amorphous regions did not change considerably and became almost constant for $TiO_2$ coated silk fibers after undergoing UV-irradiation at different times. This may indicate that $TiO_2$ coated silk fibers have more stable initial structure thanks to less degraded but stiffer β-sheet

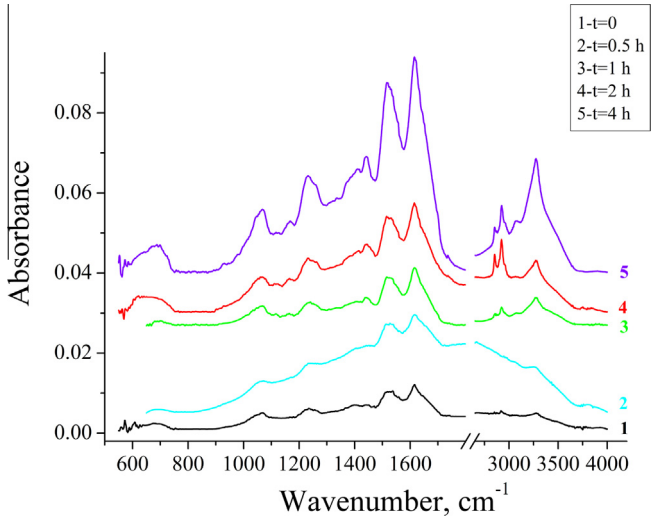



**Fig. 6.** FT-IR/ATR spectra of the uncoated silk fibers after being exposed to UV-radiation at different times (*t*).

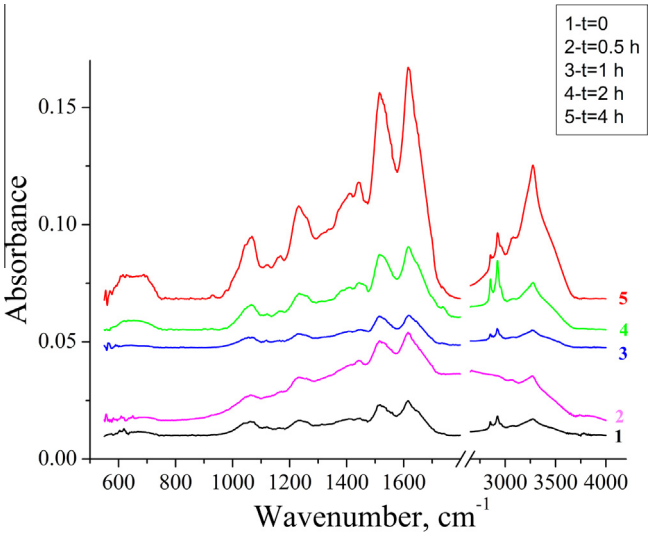



**Fig. 7.** FT-IR/ATR spectra of the $TiO_2$ coated silk fibers after being exposed to UV-radiation at different times (*t*).

**Table 4**
The ratios of the absorbance intensity of the bands to that of the standard band in the spectra of the uncoated silk fibers after the UV-irradiation.

| | Time of UV-irradiation [h] | | | | |
|---|---|---|---|---|---|
| | 0 | 0.5 | 1 | 2 | 4 |
| $A_{976}/A_{1441}$ | 0.22 | 0.15 | 0.17 | 0.16 | 0.15 |
| $A_{1000}/A_{1441}$ | 0.30 | 0.29 | 0.30 | 0.27 | 0.22 |
| $A_{1068}/A_{1441}$ | 0.86 | 0.62 | 0.70 | 0.57 | 0.54 |
| $A_{1235}/A_{1441}$ | 0.98 | 0.88 | 0.81 | 0.85 | 0.84 |
| $A_{1258}/A_{1441}$ | 0.86 | 0.81 | 0.78 | 0.76 | 0.76 |
| $A_{1516}/A_{1441}$ | 1.77 | 1.79 | 1.74 | 1.50 | 1.65 |
| $A_{1558}/A_{1441}$ | 1.39 | 1.44 | 1.35 | 1.17 | 1.30 |
| $A_{1616}/A_{1441}$ | 2.12 | 1.88 | 2.07 | 1.70 | 1.87 |
| $A_{1645}/A_{1441}$ | 1.40 | 1.35 | 1.42 | 1.21 | 1.39 |
| $A_{1616}/A_{1645}$ | 1.51 | 1.39 | 1.46 | 1.40 | 1.34 |

**Table 5**
The ratios of the absorbance intensity of the bands to that of the standard band in the spectra of the $TiO_2$ coated silk fibers after the UV-irradiation.

| | Time of UV-irradiation [h] | | | | |
|---|---|---|---|---|---|
| | 0 | 0.5 | 1 | 2 | 4 |
| $A_{976}/A_{1441}$ | 0.14 | 0.13 | 0.10 | 0.04 | 0.06 |
| $A_{1000}/A_{1441}$ | 0.33 | 0.21 | 0.23 | 0.14 | 0.17 |
| $A_{1068}/A_{1441}$ | 0.82 | 0.49 | 0.61 | 0.60 | 0.53 |
| $A_{1235}/A_{1441}$ | 0.89 | 0.83 | 0.79 | 0.77 | 0.79 |
| $A_{1258}/A_{1441}$ | 0.79 | 0.73 | 0.7 | 0.76 | 0.70 |
| $A_{1516}/A_{1441}$ | 1.82 | 1.6 | 1.81 | 1.72 | 1.76 |
| $A_{1558}/A_{1441}$ | 1.36 | 1.19 | 1.13 | 1.25 | 1.20 |
| $A_{1616}/A_{1441}$ | 2.06 | 1.71 | 1.83 | 1.83 | 1.97 |
| $A_{1645}/A_{1441}$ | 1.49 | 1.23 | 1.47 | 1.46 | 1.41 |
| $A_{1616}/A_{1645}$ | 1.38 | 1.39 | 1.24 | 1.38 | 1.40 |

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microcrystals by the effect of $TiO_2$ coating resulted in higher initial resistance to deformation with increasing dose of UV-irradiation than uncoated silk fibers.

## Discussion

The titanium dioxide coating formed as a thin layer as well as the accumulation of some excessive amount of $TiO_2$ particles on the surface of the filaments was shown by SEM images. We suppose that the formation mechanism of $TiO_2$ coating is the same as that discussed earlier for $TiO_2$ coated silk yarns [19]. That is, titanium dioxide particles interacted with OH groups of amino acids such as serine on the surface of the β-sheet microcrystals and tyrosine in the amorphous regions and $TiO_2$ network was formed on the surface of silk filaments as a result of hydrolysis and condensation reactions of the sol-gel method. During the formation of $TiO_2$ network, it is also possible that $TiO_2$ sol has interactions with the side groups of amino acids containing OH groups found in the amorphous chains and some $TiO_2$ molecules were bound to some amorphous chains. The $TiO_2$ network formed especially on the surface of the β-sheet microcrystals increased considerably the stiffness and Young's modulus of silk filaments by 17%. It also increased slightly other tensile properties such as the tensile strength, breaking extension, and work of rupture related with the toughness of the silk filament. On the other hand, the UV-radiation affected greatly not only the stress-strain curves but also the tensile characteristics of both uncoated and $TiO_2$ coated silk filaments. Quite important observation on the stress-strain curves of the irradiated uncoated and $TiO_2$ coated silk filaments is the disappearance of the yield point and of the plastic deformation region after the time of UV-irradiation higher than 1 h. It is known that at around the yield point or past the yield point, especially flexible chains in amorphous regions are extended and inter-chain hydrogen bonds between polypeptide chains are broken, thus, for further extension, these chains are extended and slide and past each others or crystalline regions; therefore, this process will result in plastic deformation. It is also known that flexible amorphous chains have a much greater contribution to the extensibility of the silk fiber rather than that crystalline region does. Thus, it can be supposed that photodegradation and destruction in macromolecular chains of amorphous regions occurred more intensively than those in crystalline regions, hence, more amorphous chains were damaged and broken which could not contribute to extensibility of the silk filaments. Therefore, the silk filaments regardless of being uncoated or coated with $TiO_2$ after being irradiated under UV-light for the time higher than 1 h could not extend much and show a plastic deformation.

After the application of UV-radiation with high intensity, tensile properties of both uncoated and $TiO_2$ coated silk filaments decreased greatly with a similar tendency as the time of irradiation increased up to around 4 h. However, $TiO_2$ coated silk filaments showed a slightly changed but significantly higher Young's modulus as a prominent tensile characteristic than that of corresponding value of the uncoated silk filaments. It is known for the uncoated silk fibers that in general, β-sheet crystalline regions thanks to their high stiffness and modulus, contribute greatly to the strength and Young's modulus of the fibers, therefore they cannot contribute to the extensibility of the fibers greatly. On the other hand, flexible amorphous chains contribute to the extensibility of the silk fibers more than crystalline regions do. In the case of $TiO_2$ coated silk fibers, it is supposed that $TiO_2$ molecules form a network on the surface of the silk filaments, especially on the interacting surfaces of the β-sheet microcrystals by forming covalent bonds with oxygen atom of the side groups of the OH containing amino acids such as serine in the β-sheet microcrystals and between each others via O-Ti-O bonds. Hence, this strong $TiO_2$ network makes the β-sheet microcrystals stronger and stiffer which contributes to the Young's modulus significantly. Such $TiO_2$ network also has a protection against UV-radiation. The reason why the breaking extension decreased dramatically for the uncoated and $TiO_2$ coated silk filaments after UV-irradiation is that amorphous chains could not be extended and broken easily due to sever photodegradation on these chains. Besides, the initial resistance and protection against UV-radiation was also shown after the $TiO_2$ coated silk fibers were exposed to UV-radiation for 1 day. That is, although the uncoated silk fibers could not be tested at all after the 1 day UV-irradiation, $TiO_2$ coated silk filaments showed remarkably a stress-strain curve in the initial elastic region with the Young's modulus being 13 GPa. It can be supposed that $TiO_2$ coating has an important and considerable contribution to the crystalline regions and their stiffness; hence, it provides a more protective effect on the crystalline regions against photodegradation and photodestruction. Therefore, less photodegradation can be supposed in the β-sheet crystalline regions than in amorphous regions. This can be also supported with the result that the ratio of the crystalline to amorphous regions of $TiO_2$ coated silk fibers did not change much and became almost constant with increasing time of UV-irradiation. It indicates a more stable and less photo degradable crystalline regions.

Moreover, the FT-IR/ATR spectral analysis clearly revealed that great photo degradation occurred in not only crystalline regions but also in flexible amorphous chains. The decrements in the absorbance ratios of the bands at 976 and 1000 $cm^{-1}$ indicated the deformation and degradation on the $CH_3$ groups and $C_\alpha$–$C_\beta$ stretching vibrations in the β-sheets. It was shown with the changes in the absorbance intensity of the band at around 1068 $cm^{-1}$ that some $C_\alpha$–C bonds in β-sheet crystalline regions were greatly deformed. Besides, the decrease in the intensities of the amide bands from amorphous regions indicates also deformation and degradation in amorphous regions as well as β-sheet crystalline regions. It could not be clearly deduced from the absorbance ratios where the photodegradation takes place more intensively, but the absorbance ratio of crystalline to amorphous region ($A_{1616}/A_{1645}$) indicates that the $TiO_2$ coated silk filaments obtained more stable initial structures thanks to stiffer β-sheet microcrystals by the effect of $TiO_2$ coating, whereas the ratio in the uncoated silk filaments decreased with increasing time of the UV-irradiation which may indicate a slight decrease in the crystallinity owing to probably more photodegradation in the crystalline regions.

## Conclusions

The results of the uniaxial tensile tests revealed that after the silk filaments were coated with titanium dioxide, especially Young's modulus increased greatly by around 17% and the work of rupture which is related with the toughness increased 11%, whereas tensile strength and breaking extension slightly increased, i.e., 3% and 6% respectively. After the application of UV-radiation with high intensity, as the time of UV-irradiation was increased, most of the tensile properties of both uncoated and $TiO_2$ coated silk fibers decreased dramatically except for Young's modulus which did not change much. It was shown that even though the Young's modulus of both uncoated and $TiO_2$ coated silk filament changed slightly after time of UV-irradiation equaling 1 h and ultimate tensile strength decreased by only around 18% and 23%, for uncoated and $TiO_2$ coated silk filaments, respectively, breaking extension decreased greatly by 67% and 72%, and work of rupture decreased by 87% and 90% for uncoated and $TiO_2$ coated silk filaments, respectively, which implies a great photo degradation in the silk fibroin structure, especially, in amorphous polypeptide chains

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which contribute greatly to the extensibility of the silk fibers in general. It was also observed that only the Young's modulus of the $TiO_2$ coated silk filaments became a prominent characteristic which became always higher than that of corresponding uncoated silk filaments with increasing time of UV-irradiation. Moreover, great changes were seen on the stress-strain curve of uncoated and $TiO_2$ coated silk filaments with the effect of UV-radiation. That is, after the time of UV-irradiation being higher than 1 h, a yield point and plastic deformation region could not be observed which significantly indicate that a great photo degradation and destruction took place dominantly on the amorphous flexible chains.

Another important result was seen after 1 day UV-irradiation, even though the uncoated silk filaments could not show any resistance and could not be tested at all because of being very weak and brittle, the $TiO_2$ coated silk filaments showed a stress-strain curve in initial elastic region with breaking extension and stress being 0.7% and 81 MPa, respectively. The Young's modulus became ~13 GPa which implies the great contribution of stiffer but less photodegradable β-sheet microcrystals by the effect of titanium dioxide coating. The results obtained from FT-IR/ATR spectral analysis revealed that photodegradation and destruction process took place in both crystalline and amorphous regions. Significant deformation and degradation on the $CH_3$ groups and $C_\alpha$–$C_\beta$ vibration in the β-sheet microcrystals were seen. Also the decrease in the absorbance ratio of the band assigned to $C_\alpha$–C stretching vibration in the backbone of the polypeptide chains in β-sheets were observed. Decrease in the absorbance ratios of the Amide I, II, and III bands for both crystalline and amorphous regions of uncoated and $TiO_2$ coated silk filaments also indicate photodegradation in both β-sheet crystalline and amorphous regions. The ratio of crystalline to amorphous regions in $TiO_2$ coated silk filaments which can be considered as almost constant value with increasing UV-irradiation time indicates that more stable β-sheet microcrystals against photodegradation and photodestruction. However, the ratio for the uncoated silk filaments considerably decreased which may indicate the decrease in the crystallinity as a result of photodegradation and photodestruction.

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