

# Effects of Solar Radiation on Collagen-Based Biomaterials

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The effect of solar radiation on collagen and collagen/synthetic polymer blends in the form of thin films and solutions has been studied by UV-VIS and FTIR spectroscopies. Films and solutions of collagen blended with poly(vinyl alcohol) (PVA) and poly(vinyl pyrrolidone) (PVP) were irradiated by solar light. It was found that UV-VIS spectra, which characterize collagen, collagen/PVA, and collagen/PVP blended films, were significantly altered by solar radiation. FTIR spectra of collagen, collagen/PVA, and collagen/PVP films showed that after solar irradiation, the positions of Amide A bands were shifted to lower wavenumbers. There was not any significant alteration in the position of Amide I and Amide II bands of collagen and its blends after solar radiation. The effect of solar UV radiation in comparison with artificial UV radiation has been discussed.

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## 1. INTRODUCTION

Sunlight is a key environmental factor in almost all ecosystems, and the many ecological effects of visible and infrared wavelengths have been well established. In living organisms sunlight has a profound effect on premature skin aging and skin cancer [1–3].

Naturally occurring polymers absorb solar radiation and undergo photolytic, photo-oxidative, and thermal-oxidative reactions that result in the degradation of the material [4, 5]. The degradation suffered by biopolymeric materials can lead to loss of mechanical properties which limit their performance. In sunlight mainly the ultraviolet radiation determines the useful lifetime of many materials. Any increase in the UV content in terrestrial solar radiation due to a partial depletion of the stratospheric ozone layer is therefore expected to have an impact on biopolymers. Moreover, for biopolymers the humidity and air temperature during exposure have a large influence on photodegradation. For synthetic polymers it was found that the samples kept at the lower temperatures deteriorated much more slowly than those at ambient temperature although both were exposed to the same dose of solar radiation [5].

Collagen is the main protein of connective tissue and the main component of skin [6–8]. It is widely used as a biomaterial, which is often exposed to solar radiation. The interaction of solar radiation with collagen in vitro has been studied previously [9]. The solar radiation altered the properties

of the collagen films and caused changes in the conformation of the collagen molecule but did not lead to the cleavage of the chain. Artificial UV irradiation can induce crosslinks into collagen fibrils. However, this is complicated by peptide bond scission events that may also occur through free radical mechanisms [10–20].

In this paper the results obtained for collagen-based materials after solar radiation at Plataforma Solar de Almería in Spain are presented.

## 2. EXPERIMENTAL DETAILS

Collagen was obtained in our laboratory from tail tendons of young albinos' rats. We used the same method of extraction as previously employed [16, 17]. After washing in distilled water these tendons were dissolved in 0.4 M acetic acid. Collagen films were obtained by casting the solution onto a glass plate or CaF<sub>2</sub> spectrophotometric windows. After solvent evaporation, samples were dried under air at room temperature.

PVA and PVP were purchased from Sigma as a powder. The powder was dissolved in 0.4 M acetic acid. Collagen/PVA and collagen/PVP blends were prepared by mixing of appropriate volumes of collagen and PVA or PVP in acetic acid such that solutions were produced containing collagen-polymer blends with a final weight ratio 1 : 1. Films were obtained by casting the solution onto glass plates or CaF<sub>2</sub>

TABLE 1: Doses of solar radiation during the specimens exposition.

Day	Hours	Global radiation ( $\times 10^6$ J/m <sup>2</sup> )	UV (A+B) ( $\times 10^4$ J/m <sup>2</sup> )	Time of irradiation (days)
03.06.2003	—	0	0	0
04.06.2003	8.50–12.50	3.3	1.8	—
04.06.2003	13.30–15.30	5.9	3.5	1
05.06.2003	9.00–12.00	11.8	6.0	—
05.06.2003	12.40–15.40	21.6	12.2	2
06.06.2003	9.20–12.40	29.8	16.0	—
06.06.2003	13.17–15.30	37.5	21.1	3
09.06.2003	9.05–12.10	44.5	24.1	—
09.06.2003	12.42–15.30	54.4	30.8	4

spectrophotometric windows. After solvent evaporation, the samples were dried in air at room temperature. The samples in the form of thin films of pure collagen, collagen/PVA, and collagen/PVP blends were irradiated under air, at a temperature range of 29–42°C, by solar radiation at the Plataforma Solar de Almería, Spain, during 6 days in June 2003. The Plataforma Solar de Almería (PSA) is the largest center for research, development, and testing of concentrating solar technologies in Europe. The intensity of solar radiation including UV-A and UV-B during the exposure was constantly measured and the values of the dose are listed in Table 1. The intensity of the incident light was measured using a global UV radiometer (KIPP & ZONEN, model CUV3), mounted on the platform tilted at 37° (same angle as the CPC reactor), which provides data in terms of incident  $W_{UV}m^{-2}$ .

The UV-VIS absorption spectra of the collagen specimens, before and after solar irradiation, were recorded with a Shimadzu spectrophotometer (Model UV-1601PC). Data collection and plotting were accomplished by the UVPC program and computer data station supplied by the manufacturer.

IR spectra were obtained using a spectrophotometer (Mattson Genesis II (USA)). Spectra were recorded by absorption mode at 4  $cm^{-1}$  intervals and 16-times scanning. The functional groups in collagen and chitosan and their corresponding chemical characteristics can be observed from FTIR spectra in the wavelength range of 4000–600  $cm^{-1}$ .

### 3. RESULTS AND DISCUSSION

The absorption spectra of collagen films reveal absorption in the 250–280 nm region. The UV-VIS spectra that characterize the collagen films were significantly altered after global solar irradiation (Figure 1); irradiation of the collagen films by solar radiation leads to a minor increase in overall absorption, most notably between 240 and 300 nm. This may be due to the changing optical properties of the collagen films. We know that artificial UV irradiation causes changes in the conformation of the collagen molecule (i.e., helix-coil

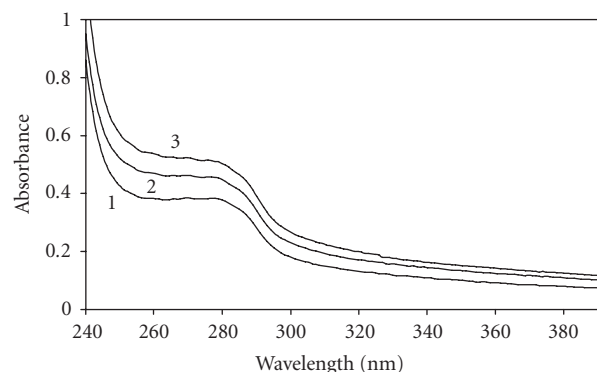


FIGURE 1: UV-VIS spectra of collagen before (curve 1) and after 2 days (curve 2) and 5 days (curve 3) of solar irradiation (key to time and dose of solar radiation in Table 1).

transition). The increased presence of a coil structure and the progressive loss of the helical character of collagen bring about an overall increase in the scattering level of the sample [15–17]. We can hypothesize that solar radiation containing UV-A and UV-B (see Table 1) also cause changes in conformation of the collagen molecule. After irradiation, the peak at 275 nm is less well pronounced, becoming more shoulder-like with increasing irradiation time. Solar radiation altered also the optical properties of collagen/PVA and collagen/PVP films. The changes of absorbance at 275 nm with dose of solar irradiation for collagen and its blends with other polymers are presented in Figure 2 (doses are listed in Table 1). The values of absorbance at 275 nm have been chosen for comparison of alterations by solar irradiation of collagen and its blends with other polymers. The absorption spectra of synthetic polymers usually do not show clear, characteristic peaks in the UV-VIS region.

For collagen/PVP blends (ratio 1 : 1), there is only a smaller difference between absorption/scattering after irradiation than in pure collagen. For the blends of collagen/PVA the alterations in UV-VIS spectra are bigger than that of pure

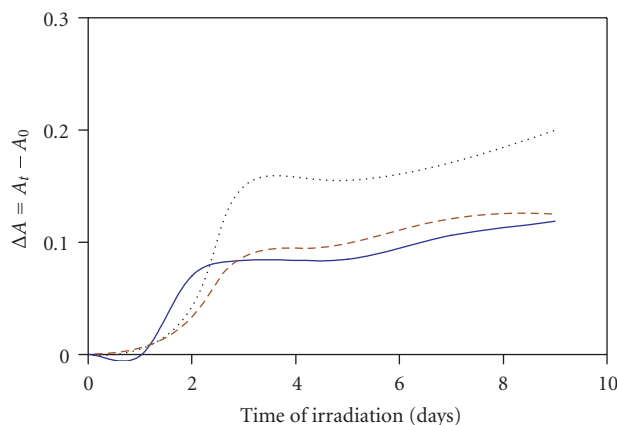


FIGURE 2: The changes of absorbance at 275 nm during solar irradiation of collagen (solid curve), collagen/PVA (dashed curve), and collagen/PVP films (dotted curve), key to time and dose of solar radiation in Table 1.

TABLE 2: The position of Amide bands in FTIR spectra of collagen and collagen-based biomaterials after solar radiation (key to time and dose of solar radiation in Table 1).

Sample	Characteristic bands ( $\text{cm}^{-1}$ )					Time of irradiation (days)
	Amide A	Amide B	=CH <sub>2</sub>	Amide I	Amide II	
Collagen	3315	3079	2936	1638	1547	1
	3311	3071	2935	1634	1546	2
	3309	3078	2940	1635	1547	3
	3301	3064	2950	1634	1547	4
Collagen/PVP blend	3329	3087	2935	1644	1550	1
	3322	3088	2953	1644	1550	2
	3323	3086	2953	1645	1551	3
	3321	3091	2955	1641	1549	4
Collagen/PVA blend	3316	3079	2937	1642	1548	1
	3321	3067	2937	1642	1550	2
	3318	3080	2938	1648	1549	3
	3310	3088	2928	1641	1548	4

collagen. This phenomenon is connected with miscibility of the components of the blend. Collagen and PVP are miscible [21], so the blend is homogenous. However, collagen/PVA are immiscible, so the film obtained from the blend is not completely transparent [22]. In artificial conditions during irradiation with UV light emitting 254 nm usually the blends were less photochemically stable than pure collagen [23]. However, artificial UV light was very energetic and its energy was sufficient to break chemical bonds. It has been found before that the presence of synthetic polymer in collagen material alters both the photochemical stability of collagen and the processes which lead to the increase of absorption, that is, photodegradation (with scission of bonds in the main chains) or phototransformation (with changes in the conformation of collagen molecules) [24].

The FTIR spectra of collagen are shown in Figure 3. Infrared spectra of proteins and synthetic polypeptides indicate a number of bands attributed to peptide bonds. They may be useful for further characterization of the bound water molecules and hydrogen bonds in collagen. Their positions are sensitive to changes in the conformation of the molecules [24]. The Amide A band of collagen (associated with the NH-stretching frequency) is usually found at 3315–3330  $\text{cm}^{-1}$ ; the Amide B band at 3080  $\text{cm}^{-1}$ ; the Amide I band at 1630–1650  $\text{cm}^{-1}$ ; and the Amide II band is centered in the range of 1530–1555  $\text{cm}^{-1}$ . The Amide II band has a complex nature with its vibrational source in both the NH deformation and CN stretching modes. In our sample the Amide A band is shifted after solar irradiation to lower wavenumbers, but Amide I and II are not altered (Table 2). These shifts

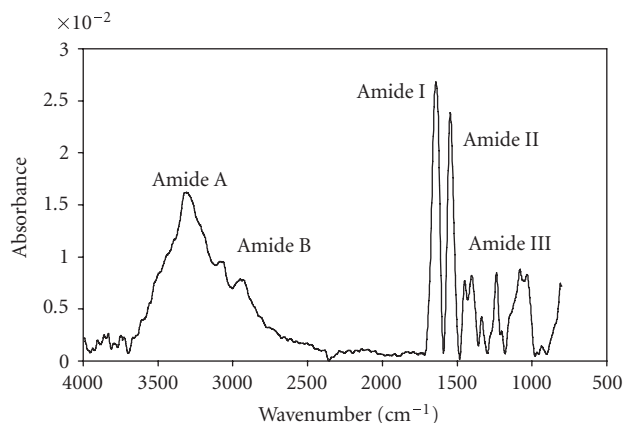


FIGURE 3: FTIR spectra of a collagen film. The Amide A, B, I, and II bands appear shifted to lower wavenumbers after solar irradiation.

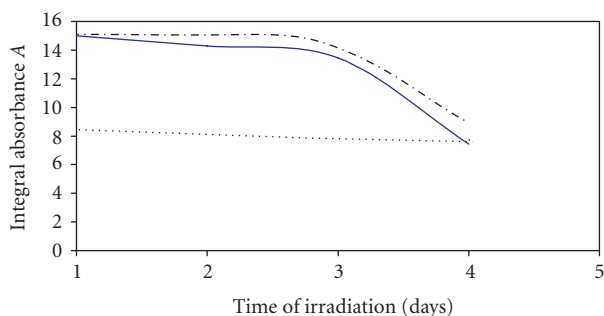


FIGURE 4: The changes of integral absorbance of Amide A band in FTIR spectra during solar irradiation of collagen (solid curve), collagen/PVA (dotted curve), and collagen/PVP films (dashed-dotted curve), key to time and dose of solar radiation in Table 1.

are possibly due to the scission of hydrogen bonds on exposure to UV irradiation from the sun: hydrogen bonds are necessary to maintain the helical structure of collagen. Similar shifts were observed for collagen/PVP and collagen/PVA blend (Table 2).

Using the FTIR method the changes in integral absorbance of the Amide bands after an extended period of solar irradiation were observed. Integral absorbance of Amide bands A, I, and II in collagen decrease after UV irradiation (Figures 4, 5, and 6). Integral absorbance for Amide A band collagen/PVA is nearly unchanged after UV irradiation (Figure 4). For collagen/PVP blends the changes of integral absorbance of Amide bands (A and II) are similar to those for pure collagen (Figures 4 and 6). The integral absorbance of the Amide A band at  $3315\text{--}3330\text{ cm}^{-1}$  is much smaller after solar irradiation than for nonirradiated specimens. This fact points out that water is loosely bound to the collagen films (Figure 4).

Structural changes on the surface of collagen films were induced by solar irradiation. These have been observed using the microscope. The microphotographs of nonirradiated and

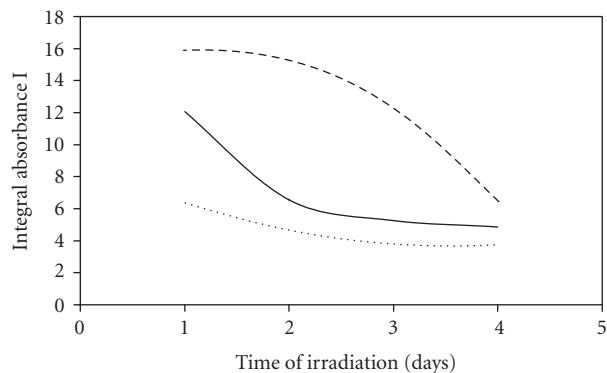


FIGURE 5: The changes of integral absorbance of Amide I band in FTIR spectra during solar irradiation of collagen (solid curve), collagen/PVA (dotted curve), and collagen/PVP films (dashed curve), key to time and dose of solar radiation in Table 1.

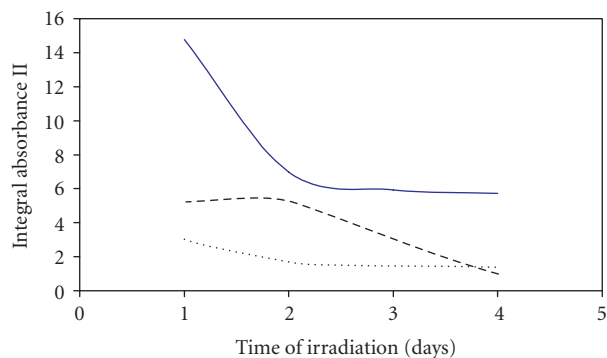


FIGURE 6: The changes of integral absorbance of Amide II band in FTIR spectra during solar irradiation of collagen (solid curve), collagen/PVA (dotted curve), and collagen/PVP films (dashed curve), key to time and dose of solar radiation in Table 1.

irradiated collagen-based films are shown in Figure 7. Due to the irradiation by solar light being carried out in open air, the specimens were usually covered by dust and other small particles supplied by the wind. We could observe the effects of these impurities on the surface of almost all specimens. This fact disrupts our ability to discuss the effect of solar UV light directly on the surface of the specimens. However, for all the films, we observed wrinkles and microcracks after solar irradiation which was the effect of loss of water bound to collagen.

#### 4. CONCLUSIONS

The solar radiation alters the properties of collagen, collagen/PVA, and collagen/PVP films. Collagen/PVA films in the conditions of the experiment were more resistant to the action of solar radiation than collagen films. UV-VIS and FTIR spectroscopies have shown that solar irradiation causes the changes in conformation of the collagen molecule. The surface properties of thin films were altered by solar radiation. Microscopic photographs have shown that irradiated



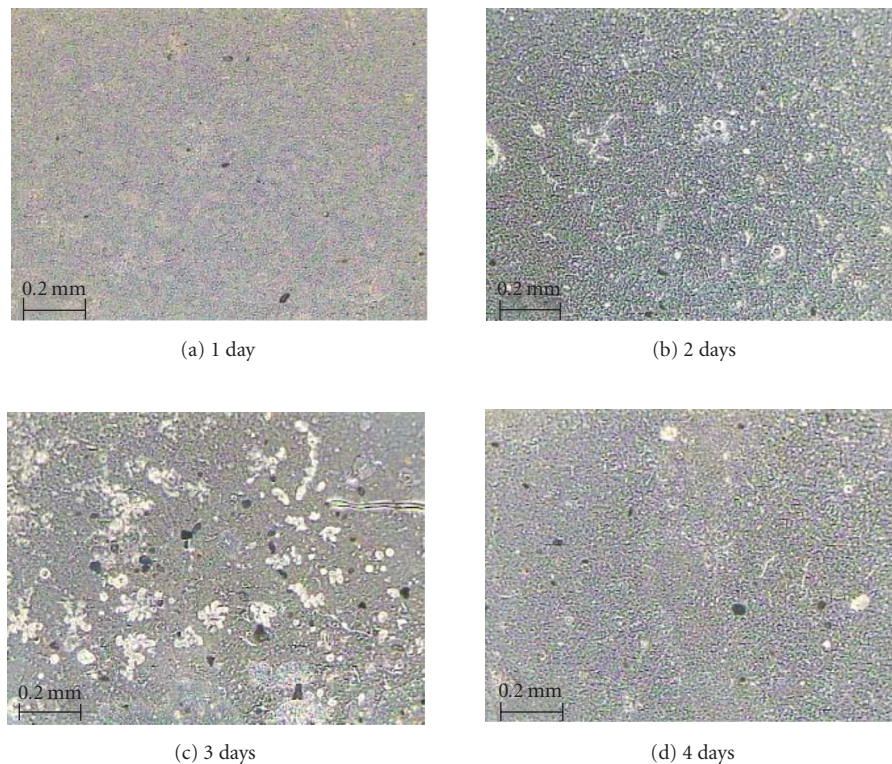


FIGURE 7: Microphotograph of collagen films before and after different doses (Table 1) of solar irradiation.

collagen films have evident surface changes. In the experiment the specimens were exposed to all wavelengths of sun light, so the effects of other components of sun light than UVB and UVA may give different effects than the artificial source of UV.

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