ACADEMIA ROMÂNĂ
*Revue Roumaine de Chimie*
http://web.icf.ro/rrch/

*Rev. Roum. Chim.*,
**2021**, *66*(1), 81-85
DOI: 10.33224/rrch.2021.66.1.09

*Dedicated to Professor Mihaela Hillebrand, on the occasion of her 80th anniversary*

# EFFECT OF UV IRRADIATION ON SURFACE OF COLLAGEN FILM

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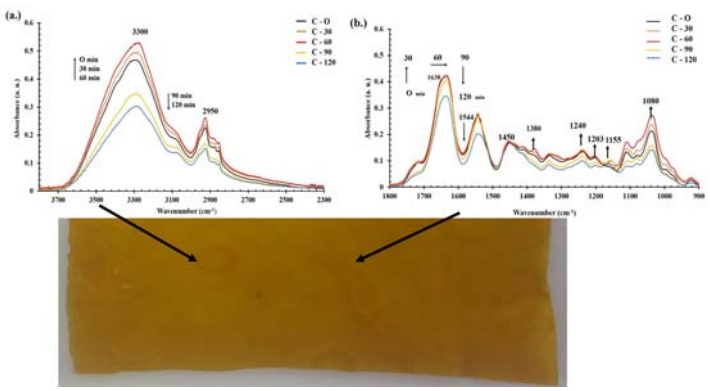
*Received September 25, 2020*

It is well known that ultraviolet (UV) radiation is considered to be one of the most hazardous external factor that induces skin damage, leading in some cases to skin carcinoma. The most abundant protein in human skin tissue is collagen, which is responsible for conferring strength and resiliency. Taking into account these aspects, the present paper study the influence of the UV-irradiation on surface of a collagen film. Thus, the surface modification of the collagen film after irradiation was monitored with the help of the FTIR spectroscopy in close correlation with contact angle determinations and profilometry analysis. The obtained data reveal that after UV-irradiation, the collagen film has shown different structural changes which depend on the irradiation time. At short irradiation time, some cleavage and reorganization of the surface structural groups take place, but in contrast, the highest radiation exposure led to degradation.




## INTRODUCTION

The sun and, implicitly, ultraviolet (UV) radiation are essential for human health, but in order to have beneficial effects we must not expose ourselves uncontrolled and unprotected. Excessive exposure to UV radiation is associated with an increased risk for various skin cancers, skin burns, accelerated skin aging, cataracts and other eye diseases.[1–3] UV radiation can in some cases decrease the effectiveness of the immune system by changing the activity and distribution of the cells responsible for releasing immune responses. Many patients have benign lesions of the dermis, others are diagnosed with skin diseases such as cancer. Therefore, diseases induced by solar radiation are practically based on the influence of UV radiation.[4]

The UV radiation from sun light it is that presently determines the useful lifetime of many materials and the organisms. The testing of biopolymers under different solar radiation is not easy, the humidity and air temperature during the exposure have a major influence on biopolymers.

Collagen is one of the main protein of connective tissue and the vital component of the

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skin. It is a vital component for skin elasticity and its degradation leads premature aging.[5,6]

The solar radiation is absorbed by the natural polymers, and can induces intra and inter-chain interaction on surface, leading to various phenomena such as, photolytic, photo-oxidative and thermal-oxidative reactions.[7,8] The interaction of UV radiation with collagen in vitro may provide information about the effects of its influence in vivo. Moreover, collagen is a protein widely used as a biomaterial, which is often exposed to solar radiation.

The aim of this paper was to investigate the photochemical changes in the structure of collagen surface film before and after UV-irradiation by using FTIR spectroscopy and the surface properties such as wettability (contact angle determination) and roughness (profilometry analysis). These investigation give valuable information about the influence of the UV radiation on the collagen structure and could be correlated with the damage of the sun light on the skin surface.

## EXPERIMENTAL

### Materials

The experiments were realized using a UV medical lamp (V-TAC-38W), which provide a continuous radiation at 254 nm and 38 W. 3 x 3 $mm^2$ collagen films were placed at 20 cm from the UV lamp and irradiated at different time: 30, 60, 90, 120 min. The samples were denoted from C-0 to C-120, where C represent collagen and 0-120 are the irradiation time.

In order to obtain collagen film, 10 mg/ml collagen type I (Sigma-Aldrich (99, 85%)) were dissolved in phosphate buffer at pH = 3 and mixed for 2 hours for homogenization. Then, the solution was cast on a silicon slide and left to dry at 37 $^oC$ for two days.

### Characterization methods

The static contact angle determinations were carried out by using a CAM 101 Optical Video Contact Angle System (KSV Instruments LTD, Finland) equipped with a Hamilton syringe, video camera and drop shape analysis software. MilliQ water and ethylene glycol were used as solvents for these studies and for each kind of liquid, five different regions of the surface were selected to obtain a statistical result. A stylus profiler - Tencor Alpha-Step D-500 (KLA Tencor Corporation, Milpitas, CA, USA) was used to measure the average square roughness ($R_a$) parameters of samples with a speed of 0.10 mm/s. The measurement was made with a loading force of 15 mg and achieved an interval of filtration about 0.060 mm.

Attenuated Total Reflection - Fourier Transform Infrared (ATR-FTIR) analysis was performed by using The infrared spectra were investigated using a LUMOS FT-IR Microscope (Bruker Optik GmbH, Ettlingen, Germany) software for spectral processing OPUS 8, in the range 600-4000 $cm^{-1}$, 64 scans were taken for every spectrum, with a baseline correction.

## RESULTS AND DISCUSSION

The effect of the UV-irradiation on the collagen film surfaces was monitored by static contact angles (θ) measurements using the sessile-drop method. The values can be used to estimate the wettability and surface tension of a solid surface. The static contact angles were measured before and after UV-irradiation by using two test liquids – milli Q water (W) and ethylene glycol (EG). The obtained values are illustrated in Table 1. As is observed, the wettability varies with the irradiation time and is different due the various interactions/ reorganizations/degradations that occur at the irradiated collagen surface.

*Table 1*

Static Contact angle values determined on collagen films before and after exposure to UV radiation

| SAMPLES | Contact angle ($^o$) | | $W_a$ (mN/m) | $\gamma_{SV}$ (mN/m) | $\gamma^P_{SV}$ (mN/m) | $\gamma^d_{SV}$ (mN/m) | $\gamma_{SL}$ (mN/m) | Roughness Ra (nm) |
|---|---|---|---|---|---|---|---|---|
| | W | EG | | | | | | |
| **C-0** | 82.7 | 60.6 | 82.05 | 26.8 | 8.84 | 17.97 | 17.56 | 416 |
| **C-30** | 79.4 | 66.44 | 86.24 | 25.55 | 17.32 | 8.22 | 12.11 | 435 |
| **C-60** | 72.6 | 62.3 | 94.63 | 31.19 | 25.06 | 6.13 | 9.37 | 440 |
| **C-90** | 92.9 | 48.75 | 69.11 | 55.58 | $7.93 * 10^{-5}$ | 55.58 | 58.26 | 303 |
| **C-120** | 54.08 | 26.83 | 115.90 | 82.48 | 80.71 | 1.77 | 14.6 | 350 |

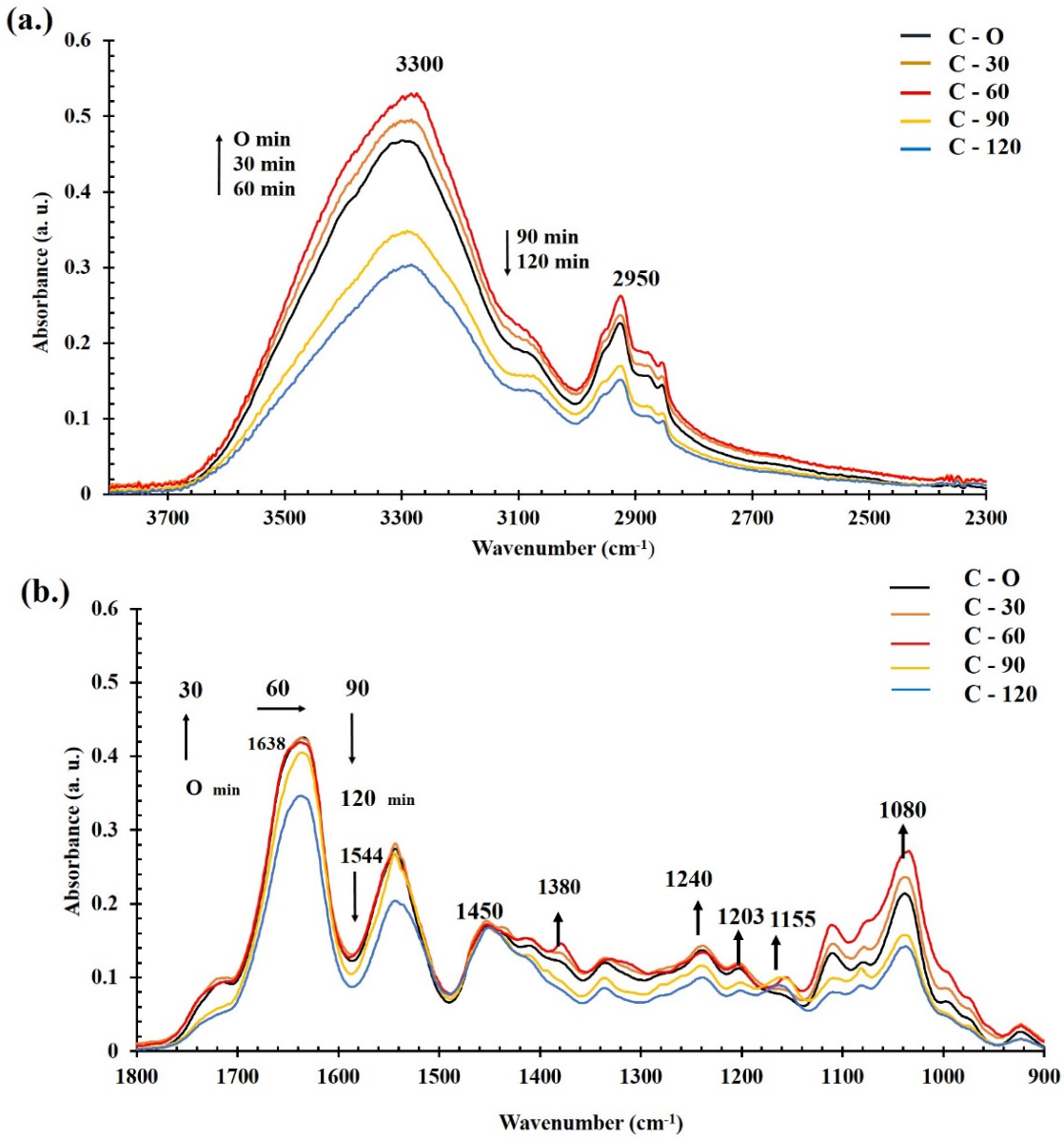


Fig. 1 – ATR-FTIR measurements in the 3700–2300 $cm^{-1}$ (a.) and 1800-900 $cm^{-1}$ (b.) range for collagen film before and after UV-irradiation at different time.

By using the measured contact angle values and Owen–Wendt method, some surface parameters (surface free energy, adhesion work, interfacial solid-liquid tension) could be calculated.[9] The results are shown in Table 1. The value of surface free energy of collagen film before UV-irradiation (26.8 mN/m) is smaller than at 60 and 120 min irradiated films (31.19 mN/m and 82.48 mN/m, respectively). At 30 min of irradiation, a decrease of the surface energy is observed (25.55 mN/m). This phenomenon is probably due to the appearance of some groups with oxygen at the surface after oxidation in the first stage of UV-irradiation.

After 90 minutes of irradiation, the surface energy increases (55.58 mN/m), due to the increase of the contact angle value. This can be explained by the fact that at this stage, the surface presents an important crosslinking effect due to the degradation with replacement of the hydrophilic groups. It is also observed that the polar component of the surface energy has a very small value ($7.93*10^{-5}$ mN/m) compared to other values.

This behavior is also observed at the data obtained from profilometry analysis, when the roughness is the smallest (303 nm) after 90 min of UV-irradiation. The interfacial tension ($\gamma_{SL}$) can also be calculated from the contact angle values. These values could be high or low, depending on the attractive forces between molecules of the liquid and solid. Thus, there are two phenomena

that occur at the surface of collagen film after UV-irradiation. The first consists in the arising of some oxygen-containing groups at the surface of collagen film, followed by degradation and crosslinking.

The increase of polarity of surfaces suggests that oxidation took place on their surface and free radicals are formed during UV-irradiation, which may react with atmospheric oxygen leading to the formation of different oxygen containing groups (carbonyl, hydroxyl, hydroperoxide groups).[10]

The surfaces of the irradiated collagen films were also investigated through the ATR-FTIR spectroscopy. Particular attention was given to the amide bands, that are useful for structural analysis of changes on collagen films and hydrogen bonding:[11,12] amide A (3400–3350 $cm^{-1}$, assigned to -NH and -OH – stretching vibration), amide B (3085–3070 $cm^{-1}$), amide I (1700–1600 $cm^{-1}$, corresponding to C=O stretching vibrations preponderantly from protein amide), amide II (1600–1500 $cm^{-1}$, assigned to 60 % -N-H bending vibrations and 40% contribution of -C–N stretching vibrations ), amide III (1200-1300 $cm^{-1}$, corresponding to C–N stretching and N–H in plane bending from amide linkages peak; this vibration overlaps with that of wagging vibrations from -$CH_2$ groups from the glycine and proline side-chains). The absorption spectra of the UV-irradiated collagen films are present in Fig. 1((a.) and (b.)).

The frequencies that appear in FTIR spectra at 3300 $cm^{-1}$ concerns the amide A band (stretching vibrations of N–H group), which in the collagen film, are higher in comparison with the spectra of other proteins. The band position is shifted to lower wavenumber, as a result of the formation of a hydrogen bond network.

The collagen surface changes after UV-irradiation are better highlighted in the absorption spectra from Fig 1 (a). Thus, it is observed an increase of the absorbance intensity from amide A at 30 and 60 min, and a decrease at 90 and 120 min exposure time.

The same evolution has the band from 2950 $cm^{-1}$ which correspond to the $\nu_{as}(CH_2)$ stretching vibration. This behaviour could be explaining by two processes: the removal of water molecules from film and another one, the depolymerisation by removing amino and carboxyl groups, which are localized on surface. All spectra were normalized from absorption band at 1450 $cm^{-1}$ of collagen untreated film we watched the changes in intensity bands for all irradiation times (Fig. 1(b.)).

At low irradiation times, the peak at 1555 $cm^{-1}$ corresponding to the amide group in the triple helix state, is relatively constant, but after that, it has a decreasing trend. This was attributed to the cross-linking and degradation of collagen structure. Same evolution can be predicted for the absorption of the bands at 1648 $cm^{-1}$ attributed to amide I, at 1380 $cm^{-1}$ attributed to $\delta(CH_3)$ symmetric band, at 1240 $cm^{-1}$ attributed to $\delta(CH_2)$ wagging, ν(CN) amide III disordered, at 1203 $cm^{-1}$ attributed to tyrosine and phenylalanine (molecular chain) and at 1080 $cm^{-1}$ attributed to ν(CC) skeletal, random conformation. The ratio A(amide $II_{1548}$)/ A($imide_{1450}$) was modified to an increase in free -$NH_2$ groups,[13] from 1.68 to 1.83 at 30 min and to 1.9 at 60 min. After 90 min of irradiation, this ratio presents a decrease up to 1.8 and up to 1.69 at 120 min due to the beginning of the degradation.

The $A_{1240}$ / $A_{1450}$ (changes in the triple helical conformation) ratio decrease from 0.98 at 30 min until 0.83 at the last time of the UV-irradiation. The collected data suggest different changes that occurs at the irradiated collagen surfaces. Thus, at the beginning, a reorganization of the collagen chains after cleavage of the macromolecular chains under UV-irradiation was observed.

As the irradiation time increases, water evaporation and a crosslinking process take place, followed by degradation. In the latter case, the changes are irreversible.

## CONCLUSIONS

In our study, we demonstrated the harmful effect of UV radiation on a collagen film for a long time of exposure. By extrapolating this experiment to human skin, prolonged exposure to UV radiation, even if it is not solar, can induce various skin diseases. The first mechanism involves an attachment of oxygen-containing groups to the cleaved surface chains of collagen. The second mechanism is determined by the involvement of the new arising groups and the collagen groups leading to a crosslinking phenomenon followed then by a degradation process.

*Acknowledgements.* The authors acknowledge the financial support of this research through the Project "Partnerships for knowledge transfer in the field of polymer materials used in biomedical engineering" ID P_40_443, Contract no. 86/8.09.2016, MySMIS 105689, cofinanced by

the European Regional Development Fund by the Competitiveness Operational Programme 2014-2020, Axis 1 Research, Technological Development and Innovation in support of economic competitiveness and business development, Action 1.2.3 Knowledge Transfer Partnerships.

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