UV, visible and IR laser interaction with gelatine

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Abstract. In this work we investigate the effects on gelatine films of nanosecond pulsed laser irradiation at different laser wavelengths from the UV to the IR at 248, 266, 355, 532 and 1064 nm. We compared gelatines differing in gel strength values (Bloom 75 and 225) and in crosslinking degree. Formation of bubbles at the wavelengths in the UV (248 and 266 nm), melting and resolidification at 355 nm, and formation of craters by ablation in the VIS and IR (532 and 1064 nm) are the observed morphological changes. On the other hand, changes of the fluorescence behaviour of the films upon UV irradiation reveal chemical modifications of photolabile chromophores.

1. Introduction
Gelatine has focussed much scientific interest due to the large number of commercial applications for edible, pharmaceutical and photographic uses [1,2]. Gelatine is a proteinaceous material composed of long chains of amino acids joined through a peptide linkage derived from the parent protein structure of collagen. Gelatine manufacture is carried out by acid (Type-A) or alkali (Type-B) pre-treatment from bones in a reproducible way. Gelatine from successive extractions has different physical and chemical properties due to the hydrolytic degradation taking place during the manufacturing process [3]. Gelatine has served in previous laser ablation studies as a model substance for collagenous or soft biological tissue due to their similar light absorption properties [4-9]. However, the structural complexity of gelatine and other hydrated materials hinders the clear understanding of their interactions with laser radiation. Precise laser ablation of organic substrates, such as polymers, and biopolymers, requires using highly absorbed wavelengths to ensure energy deposition in a thin superficial layer and therefore a precise control, both lateral and in depth, of the amount of material removed. At the same time, for implementing laser processing procedures, assessment of side effects or alterations, both photochemical and morphological, induced upon irradiation is highly needed [4,10]. In the case of gelatine and collagen, previous laser ablation studies have concentrated in the mid infrared region, including the Er:YAG laser at 1.3 μm [7] where these materials absorb efficiently, and free-electron laser in the 2-10 μm region [5,8]. Tsunoda et al. characterized the materials ejected by excimer laser ablation of hydrated collagen [6]. More recently Lazare et al. [9] have reported on the surface microfoaming of collagen and other biopolymers by KrF laser ablation.

In order to gain better insight into the mechanisms that contribute to the laser ablation of gelatine, in this work we investigate the effects of pulsed laser irradiation at different laser wavelengths from the UV to the IR and their dependence with the structural properties of the gelatine substrate (Bloom value or gel strength and crosslinking degree which modifies mechanical properties). The morphological modifications induced by laser irradiation were monitored by optical and environmental scanning electron microscopy (ESEM). Laser-induced fluorescence (LIF) spectra of the irradiated areas were also measured and compared with those of virgin gelatine.
2. Experimental

Gelatines with different and well controlled physical and chemical characteristics were used for this study. Self standing films of 40 μm were prepared using aqueous solutions (6.67 wt %) of gelatine (Aldrich) and solvent evaporation at 37°C. Type-B gelatine with two different gel strength values Bloom 225 (B225) and 75 (B75) were used. The corresponding relative viscosities in water solution 6.67% wt/wt and molecular weigh (M_W) average are 5.50 cP and 77.3 kDa (B225) and 3.64 cP and 62.3 kDa (B75) [10]. B225 crosslinked films were prepared by immersion in a 4 % aqueous solution of formaldehyde followed by repeated washing in distilled water and drying at 37 °C until constant weight. Characterization of the crosslinking degree was done by weighting discs of 8 mm diameter before \((W_0)\) and after \((W_t)\) immersion in water at 37 °C under stirring at predetermined time intervals. Crosslinked gelatine films reached a steady-state of swelling within one hour. The weight swelling ratio \(q= (W_t /W_0)\) [11] resulted in values of 7.53 and 4.04 by crosslinking for 1 and 24 hours, respectively. The moisture content of the gelatine films was determined by thermogravimetric analysis on a Perkin-Elmer TGA 7. It resulted in a value of 4% in all the tested materials.

Laser irradiation of the gelatine films was carried out with the fundamental and harmonics (1064, 532, 355 and 266 nm) of a Q-switched Nd:YAG laser (pulses of 6 ns) and with a KrF excimer laser (248 nm, pulses of 20 ns). The Nd:YAG beam had a circular cross-section of 7 mm diameter with Gaussian profile while the excimer laser beam had a rectangular cross-section of 10 x 30 mm². The fluence of irradiation was determined by measuring the pulse energy with a ED-200 Gentec joulemeter and the surface of irradiation of the print left by the laser on an unplastisized PVC sheet. The morphology of the irradiated areas was inspected using an ESEM (Philips XL30). LIF of the films upon excitation at 266 nm was measured with a 0.30 m spectrograph (TMc300 Bentham)-intensified charged coupled detector (Andor Technologies, Model 2151) system.

3. Results and discussion

The absorption spectra of films of the four gelatine types were recorded with a spectrometer (Perkin Elmer, Lambda 35-UV/VIS) in the full spectral range to determine the effective optical absorption coefficients α at the irradiation wavelengths. Absorption below 240 nm can be attributed to the non-aromatic amino acids of the gelatine structure, whereas at longer wavelengths, in the 250-350 nm range, absorption is due to aromatic amino acids [3]. Negligible absorption is measured at longer wavelengths above 760 nm where water is the main chromophore. At 266 nm the derived optical penetration depths, \(1/\alpha\), of gelatine B75 and B225 are 30 and 55 μm, respectively.

At each irradiation wavelength the modification threshold fluences of the films were determined by measuring the minimum fluence that resulted in morphological alteration of the surface as observed under the ESE microscope. At 266 nm these are higher in gelatine B75 (0.5 J/cm²) than in B225 (0.2 J/cm²) despite the lower M_W, viscosity and higher absorption coefficient of the former. The thresholds for B225 crosslinked gelatines are similar to those of the B75 type. The observed threshold fluence trend is to increase with laser wavelength. For B225 the threshold increases from 0.1 J/cm² to 34 J/cm² at 1064 nm.

Figure 1 shows ESEM micrographs of the irradiated areas of the gelatine films. At 248 nm no etching is observed, even at fluences well above the modification threshold. On the contrary, the irradiated area swells by forming a foam-like layer, its thickness and the size of the bubbles depending on the fluence and the type of gelatine. For B225 irradiated at 1.9 J/cm² the foam layer thickness is about 9 μm. Sub micron size bubbles exhibit diameters ranging from 150 to 700 nm. The irradiated area of non crosslinked B gelatines displays a more regular surface than that of the crosslinked ones. Increase of fluence results in a surface with higher roughness, with many of the bubbles appearing broken at high fluence. The fact that some of the bubbles are broken but not all of them indicates that their depth from the surface varies. Development of bubbles and formation of a foaming layer, rather than etching, is also observed at 266 nm. The size of the bubbles formed at 266 nm is in the range of 1-5 μm, larger than the bubbles resulting in the irradiation of 248 nm. At a longer wavelength of 355 nm, evidence of melting and resolidification with larger size structures becomes evident. At 532 and 1064 nm, ablation craters of irregular contours are clearly observed.
More insight into the possible photochemical effects that take place in the UV irradiated areas was obtained by measuring the LIF spectra as shown in figure 2. These are similar in the four gelatine types, consisting in two broad bands centred at 290 and 450 nm. A general increase of fluorescence yield is observed and, more importantly, the relative intensity of the two bands is modified as a result of laser irradiation; for B75, the ratio $I_{450}/I_{290}$ grows from 1.3 to 3.2; for B225 this increase is somewhat reduced. Liu et al. [12] have described intrinsic fluorescence from gelatine as consisting of two bands, in similar positions as the ones reported here, which were assigned to the aromatic amino acid residue tyrosine (short wavelength) and its dimeric species bityroisine (long wavelength) formed by two tyrosine units when they are in close proximity. As demonstrated here, UV laser irradiation of gelatine films induces dramatic structural changes in the irradiated film. The mobility of the tyrosine residues may increase as a result of swelling or volume expansion, leading to a higher collision probability and the increase of formation yield of tyrosine dimers. The different $I_{450}/I_{290}$ ratio in B75 and B225 gelatines and the changes effected by UV irradiation could be correlated with differences in viscosity and mechanical strength which are higher for the latter.

![Figure 1. ESEM images of cross sections of gelatine films (40 μm thickness) after irradiation with a single laser pulse at the indicated conditions. The thickness of the foam layer shown in b) is 9 μm.](image)

![Figure 2. LIF spectra (resolution 10 nm) of gelatine films: a) B75 and b) B225. Virgin films: thin line; irradiated by 266 nm (2 J/cm²): thick line.](image)

The operational mechanisms governing the ns laser ablation of polymeric materials should be determined by the laser wavelength, the physical condition of the substrate and its water content. In UV laser ablation, electronic excitation results in the breaking of polymeric bonds through a direct photochemical process releasing gaseous products that remain trapped in the film. Thermalisation on picosecond time scales causes depolymerization and also leads to swelling [4,9]. Additionally photomechanical effects play an important role in the laser ablation of substrates with high water content, as reported by Lazare et al. [9] for collagen and other biopolymers irradiated by KrF laser. The observed microfoaming is discussed in terms of an explosive thermal mechanism assisted by the tensile component of the laser induced photoacoustic transient wave. Similarly with collagen, the high
water content of the gelatine films, reduces the tensile strength of the biopolymer and facilitates bubble nucleation and growth, together with an eventual expansion of the material in a microfoam layer as microphotographs put in evidence. The four gelatine types used herein differ in mechanical strength and viscosity. As seen here, crosslinking does not change the optical absorption of the films but enhances their mechanical properties and results in higher modification thresholds. The changes in morphology of the irradiated areas, with larger bubbles and more irregular aspect observed for the crosslinked films could be also correlated with the physical condition of gelatine. In the case of polymers, the physical parameters of the substrate (i.e. M_W) have been demonstrated to produce a dramatic effect in the ablation behaviour in low absorbing substrates [13]. The importance of the physical characteristics of the substrate is also demonstrated here for gelatine. On the other hand LIF of films has proven to be a good test of the structural/conformation changes induced by laser irradiation in the UV which results in swelling of the irradiated area and allows higher mobility of species as indicated by the increased formation of dimer fluorophores. The monitoring of dimer formation by LIF is also sensitive to differences between the various gelatine types. Dramatically different effects are observed as the laser wavelength is tuned towards the IR where the biopolymer displays negligible optical absorption. Efficient etching is observed at the longest wavelengths used in this work (532 and 1064 nm) indicating that the operative mechanisms are different from those intervening in the UV. IR laser ablation should be preferably governed by thermal mechanisms, as the photon energy is not sufficient for electronic excitation.

4. Conclusions
Single pulse irradiation with a UV laser (248 nm and 266 nm) of gelatine films produces a foaming expanded layer in contrast with the effects of melting and crater formation induced by irradiation at longer wavelengths. The comparison between gelatines with differing structural properties, i.e. gel strength (B225 and B75) and crosslinking (q= 7.53 and 4.04) reveals modifications on the laser induced microfoam. While results are discussed on the basis of thermal and photomechanical mechanisms and of the role played by the water content of the substrates, they strongly suggest the possibility of control of the laser created foaming layer.

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