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Creatinine biomaterial thin films grown by laser techniques

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Abstract Creatinine thin films were synthesised by matrix assisted pulsed laser deposition (PLD) techniques for enzyme-based biosensor applications. An UV KrF* (λ = 248 nm, $\tau \sim 10$ ns) excimer laser source was used for the irradiation of the targets at incident fluence values in the 0.3–0.5 J/cm^2 range. For the matrix assisted PLD the targets consisted on a frozen composite obtained by dissolving the biomaterials in distilled water. The surface morphology, chemical composition and structure of the obtained biomaterial thin films were investigated by scanning electron microscopy, Fourier transform infrared spectroscopy, and electron dispersive X-ray spectroscopy as a function of the target preparation procedure and incident laser fluence.

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1 Introduction

Future devices based on bioactive systems require the development of new technologies for the synthesis of biomolecular structures. The applications of biomolecular, bioactive structures are broad, including electronics, bioengineering and bio- and immuno-sensors, for clinical diagnostics and therapy, also food industry, as well as environment and safety analysis systems [1–4].

The design of these new devices require a precise control during the synthesis process chemical composition, structure, adherence and thickness of the biomaterial layers. Laser techniques, in principle, offers these advantages. In special experimental conditions the stoichiometry of complex multicomponent target molecules can be ensured during their transfer towards the substrate surface [5–8]. Moreover, the amount of material evaporated and deposited on the substrate surface can be easily controlled by the number and/or intensity of the laser pulses used for the irradiation of the targets. Nevertheless, laser radiation, due to the high intensity of the pulses, is destructive for active biomaterials, and its use in this field represents a high risk challenging objective.

Laser deposition techniques were already tested for organic polymer materials [9–11], carbohydrates as sucrose, glucose or dextran [12] and also, several biomaterials, as horseradish peroxidase and insulin [13], or bovine serum albumine [14], silk fibroin [15, 16], and fibrinogen blood proteins [17] deposition by laser techniques.

The aim of this article is to find the necessary working procedures which allow for the laser processing of biomaterials and offers then new and simple ways for design and fabrication of complex bioactive systems. We centered

our attention on creatinine (2-imino-1-methylimidazolidin-4-one), a protein produced by muscles and released into the blood. The amount produced is relatively stable in a given person. Thus, creatinine level in the serum reflects kidney function since it is determined by the rate it is being removed. Thin films of creatinine could serve as reference in future micro-sensor devices for clinical diagnostics.

2 Experimental

The thin film depositions were performed inside a stainless steel irradiation chamber. A pulsed UV KrF* (λ = 248 nm, $\tau_{FWHM} \cong$ 10 ns) excimer laser source was used for the targets' irradiation. The composite targets were prepared by dissolving creatinine ($C_4H_7N_3O$) in distilled water. The obtained solutions of 2 or 5 wt.% of creatinine were frozen in liquid nitrogen.

The laser beam was focused onto the target surface with a 30 cm FD MgF_2 lens placed outside the irradiation chamber. To avoid significant changes in the surface morphology of the targets they were rotated during the multipulse laser irradiation with a frequency of 3 Hz. The angle between the laser beam and the target surface was chosen of 45°. For the deposition of each film we applied 10^4 subsequent laser pulses, succeeding each other with a repetition rate of 2 Hz. The laser fluence on the target surface was fixed at values within the range (0.3–0.5) J/cm^2.

The irradiation chamber was evacuated down to a residual pressure of 10^{-5} Pa. The residual gases were monitored with an Amatek MA 100 quadrupole mass spectrometer. All deposition experiments were performed in vacuum.

The SiO_2 (100) quartz substrates were placed parallel to the target at a separation distance of 3 cm. Prior to introduction inside the deposition enclosure the substrates were carefully cleaned in ultrasonic bath in acetone. During the irradiation the substrates were kept at room temperature, while the frozen targets were cooled, circulating liquid nitrogen inside the rotating target holder.

The surface morphology, growth mode and thickness of the deposited creatinine thin films were investigated by scanning electron microscopy (SEM) by a XL-30-ESEM TMP device. For the elemental analysis the electron microscope was equipped with an energy dispersive X-ray (EDX) attachment. The obtained thin films chemical composition was studied also by Fourier-transform infrared spectroscopy (FT-IR). The thermogravimetry-differential thermal analyses were performed using a Perkin Elmer thermobalance, with 20–1000 °C temperature range. The atmosphere was pure nitrogen at a flow rate of 100 mL/min. The heating rate was fixed at 5 °C/min.

3 Results and discussion

The deposited thin films were investigated by FT-IR spectroscopy with the aim to obtain a first information about their molecular structure as compared to the base powder material used for the targets preparation. The FT-IR spectrum of the powder is presented in Fig. 1a, characterized by two broad bands at around 3245 and 3040 cm^{-1}, attributed to the ν_{as} NH_2 and ν_s NH_2 vibrations of standard creatinine. The spectrum of the thin film obtained 5 wt.% concentration frozen composite target and 0.3 J/cm^2 laser fluence contains the same characteristic bands (Fig. 1b).

In Fig. 2 can be observed that the FT-IR spectra of the films in the studied experimental conditions are similar. Nevertheless, the bands are less intense when the creatinine concentration in the solution used for the target preparation was reduced from 5 (Fig. 2 I) to 2 wt.% (Fig. 2 II). Moreover, the band intensity decreases also with the increase of the incident laser fluence (compare curve a with band c both for Fig. 2 I and 2 II).

The thermal profiles of the creatinine powder material are shown in Fig. 3. As indicated by the TG plateau (Fig. 3a), no weight loss occurs up to around 280 °C. At this temperature the DTA curve (Fig. 3b) has a sharp peak which indicates the onset of the decomposition. With the further increase of the temperature continuous weight loss can be observed with a further step around 600 °C. However, no significant plateau, corresponding to clearly defined intermediate products appeared during heating until 800 °C.

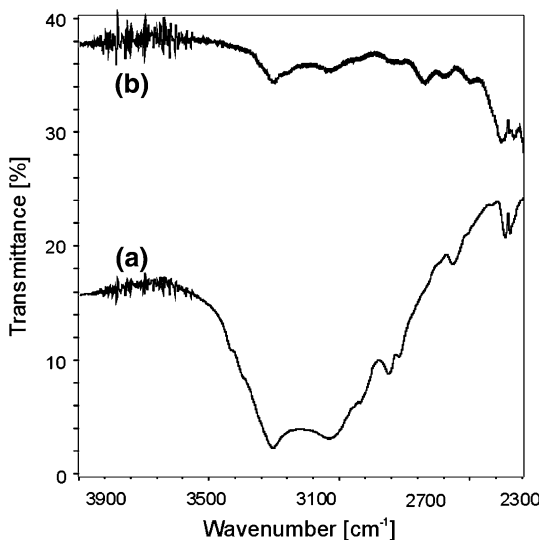


Fig. 1 FT-IR spectra of the (**a**) base creatinine powder and (**b**) thin film obtained from the 5 wt.% concentration frozen composite target

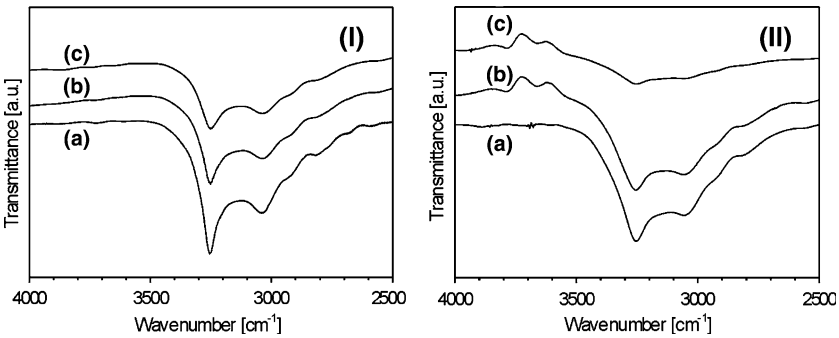


Fig. 2 FT-IR spectra of (**I**) thin films obtained from the 5 wt.% concentration frozen composite target and (a) 0.3, (b) 0.4 as well as (c) 0.5 J/cm^2 laser fluence and (**II**) thin films obtained from the 2 wt.% concentration frozen composite target and (a) 0.3, (b) 0.4 as well as (c) 0.5 J/cm^2 laser fluence

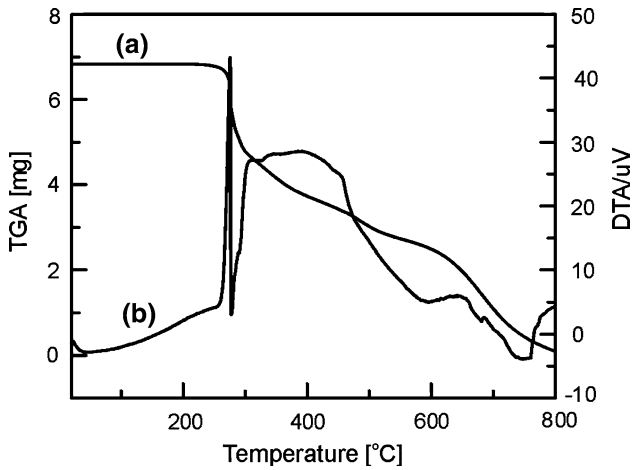


Fig. 3 (**a**) TGA and (**b**) DTA curves of the base creatinine powder

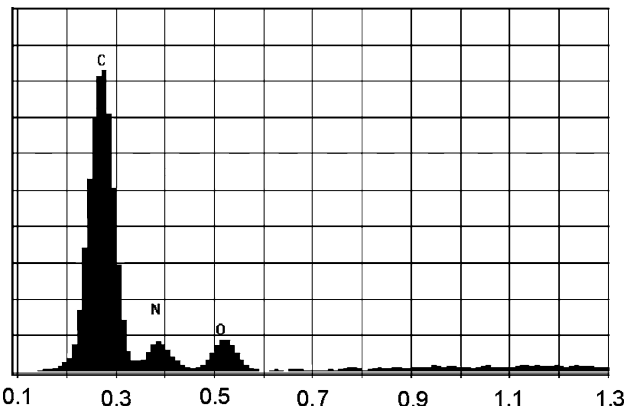
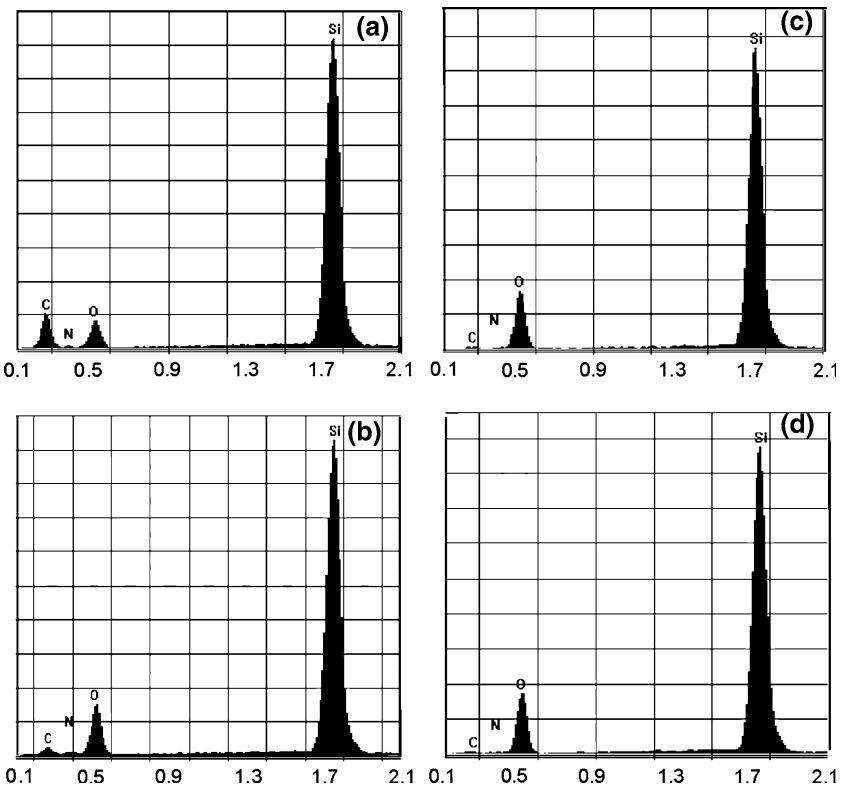


Fig. 4 EDX spectrum of the base creatinine powder

Fig. 5 EDX spectra of thin films obtained from the 5 wt.% concentration frozen composite target, (**a**) 0.3 and (**b**) 0.5 J/cm^2 laser fluence as well as 2 wt.% concentration frozen composite target, (**c**) 0.3 and (**d**) 0.5 J/cm^2 laser fluence



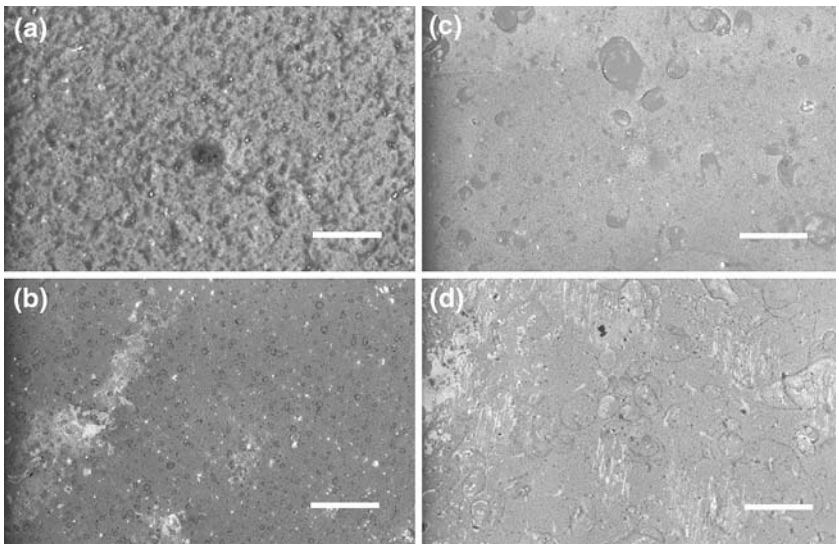


Fig. 6 Typical SEM micrographs of thin films obtained from the 5 wt.% concentration frozen composite target, (**a**) 0.3 and (**b**) 0.5 J/cm^2 laser fluence as well as 2 wt.% concentration frozen composite target, (**c**) 0.3 and (**d**) 0.5 J/cm^2 laser fluence. The mark corresponds to 200 μm for all micrographs

EDX spectroscopy was used for the further investigation of the obtained thin films chemical composition. The EDX spectrum of the creatinine powder material is presented in Fig. 4. The spectra contain the lines corresponding to carbon, nitrogen and oxygen. Hydrogen is missing since as known, elements lighter than boron cannot be detected by EDX.

The presence of the constituent elements of creatinine in the composition of the thin films was confirmed by the EDX results (Fig. 5). No other lines besides those corresponding to carbon, nitrogen, and oxygen were detected in the spectra (Fig. 5). We note that the EDX spectra can be used in this case only for qualitative analyses. Indeed, a quantitative analysis is not possible, as the oxygen K$\alpha_{1,2}$ line includes contributions both from the thin films and from the SiO_2 substrate. Nevertheless, as can be observed, the lines intensity decreases with the increase of the laser fluence (Fig. 5a, b) Moreover, lower creatinine concentration in the solution used for the target preparation lead to deposition of very low amount of material, close to the detection limit by EDX (Fig. 5c, d).

Indeed, as can be observed from the SEM micrographs (Fig. 6), the amount of deposited material decreases both with the increase of the laser fluence (compare Fig. 6a, b) and the decrease of the creatinine concentration in the solution used for the target preparation (compare Fig. 6a, c). Uniform, continuous creatinine thin films are obtained at the lowest, 0.3 J/cm^{-1} laser fluence and solutions with 5 wt.% creatinine concentration for the target preparation. The thickness of the films was evaluated from cross section SEM micrographs. Figure 7 shows the cross section SEM micrographs of the thickest films obtained at the lowest, 0.3 J/cm^2 laser fluence from the (a, c) 2 wt.% and (b, d) 5 wt.% creatinine concentration frozen composite targets. From the micrographs deposition rates of about 0.8 and 2 Å were evaluated for the films obtained from the 2 wt.% respectively 5 wt.% creatinine concentration frozen composite targets.

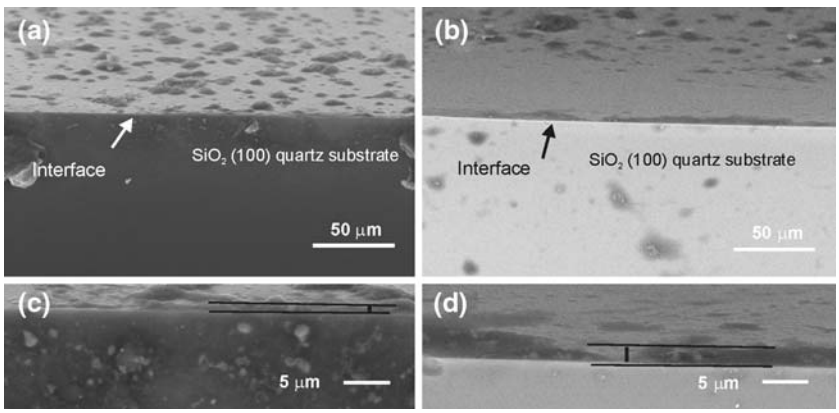


Fig. 7 Cross section SEM micrographs of thin films obtained from the (**a**, **c**) 2 wt.% and (**b**, **d**) 5 wt.% concentration frozen composite targets at 0.3 J/cm^2 laser fluence

As indicated by the thermogravimetry–differential thermal analyses, thermal decomposition of creatinine takes place already at around 280 °C. Thus, decomposition could be expected during the thin films growth, under the action of the subsequent laser pulses incident of the target. Nevertheless, as confirmed by our FT-IR results, the deposited thin films preserved the structure of the base material used for the targets preparation, representing a new evidence of the effectiveness of the frozen composite target preparation procedure used in the MAPLE technique. On the other hand, the decrease of the intensity of the FT-IR bands and EDX lines as well as the deposition of thinner films with the increase of the incident laser fluence value suggest that high laser fluences lead to the partial decomposition of the irradiated biomaterial, even if embedded in the frozen solvent matrix.

4 Conclusions

Creatinine biomolecular thin films were grown by matrix assisted pulsed laser deposition technique. A pulsed UV KrF* (λ = 248 nm, $\tau_{FWHM} \cong 10$ ns) excimer laser source was used for the irradiation of the frozen composite targets. The effect of the creatinine concentration in the frozen composites, and the incident laser fluence on the deposited films chemical composition and surface morphology was investigated by FT-IR spectroscopy, EDX and SEM. Our results indicate that there exists the possibility to find the suitable experimental conditions, frozen composites of 5 wt.% creatinine solvent, 0.3 J/cm^2 incident laser fluence, which lead to the deposition of uniform continuous thin films, with chemical composition and molecular structure identical to those of the starting biomaterial used for the target preparation.

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