Matrix assisted pulsed laser evaporation for Laccase immobilization in biosensors

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Matrix Assisted Pulsed Laser Evaporation (MAPLE) has been considered as a technique exploitable within strategies for immobilization of enzymes, because it is able to produce thin films of organic material [1].
Enzyme immobilization on the transducer is a fundamental and critical step in a biosensor design. The strategy of the immobilization greatly influence the properties of the biocatalyst in terms of activity, life-time, stability of response and cost of the resulting device [2]. Several method have been developed for enzyme immobilization [2] and MAPLE can represent a usefull alternative approach. Our previous results on the deposition of Laccase thin films by MAPLE obtained with water as the solvent were very promising [3]. Laccase was chosen since it is a redox enzyme widely used as a biological recognition component in biosensors for the detection of polyphenols [4]. In this study Laccase film was deposited by MAPLE using benzene as the solvent and modifying some deposition conditions to improve the enzymatic film performance. In table 1 are reported the deposition parameters used, in particular the values both of pulse energy and deposition time that were lower than those used for the deposition from the Laccase aqueous solution. Thin film molecular structure and surface morphology were evaluated by Fourier Transform Infrared Spectroscopy and Atomic Force Microscopy, respectively. Moreover, the enzymatic activity was determined by spectrophotometric and chronoamperometric analysis by using syringaldazine and catechol as the enzyme substrates, respectively. FTIR analysis (Figure 1) demonstrated that Laccase underwent no substantial modification in primary and secondary structures during MAPLE processes with benzene or aqueous solutions. Moreover, also AFM analysis (Figure 2) showed that Laccase thin films from both processes were slightly different and both of them covering completely the surface. Nevertheless, MAPLE with benzene by using the optimized deposition conditions has allowed to obtain more active films in a shorter time with respect to those obtained with water, as evidenced both by spectrophotometric (Figure 3) and electrochemical (Figure 4) results. Therefore, we can conclude that MAPLE with benzene can be an effective innovative technique for immobilization of redox enzymes for biosensor development, although further investigation are needed.

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Table 1 MAPLE deposition conditions.

Laser	Nd-YAG
wavelength	266 nm
pulse width	5÷7 ns
repetition rate	10 Hz
pulse energy	2 mJ/shot
spot area	1 mm^2
Target-substrate distance	35 mm
Chamber pressure	10^{-4} mbar
Deposition temperature	room temperature
Deposition time	7200 s
Target composition	1% Laccase in benzene

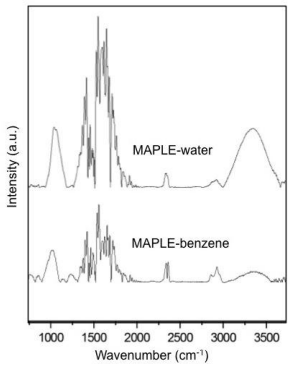

Figure 1. FTIR spectra from Laccase thin films obtained by MAPLE with water or benzene.

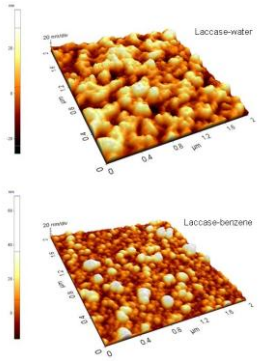

Figure 2. AFM images of Laccase thin films obtained by MAPLE with water or benzene.

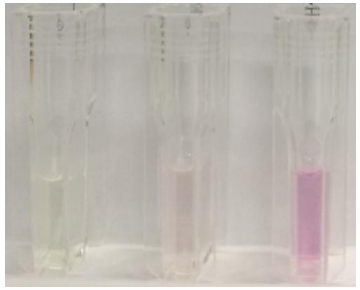

Figure 3. Colour development from activity of Laccase thin film from MAPLE by using water (B) and benzene (C), compared with the blank (A) (reaction mixture without any Laccase).

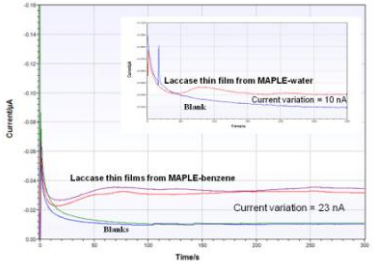

Figure 4. Chronoamperometric responses of Laccase thin film obtained by MAPLE on screen printed carbon electrode. Transients without (blank) and with the addition of 100 μM catechol. Applied potential: -0.2 V vs Ag pseudoreference.