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Immobilization of Tyrosinase in Langmuir-Blodgett (LB) films for biosensing

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Abstract: Biosensors are composed of biomolecules immobilized in solid supports, and their performance is highly dependent on the characteristics of the matrix. In this work, the enzyme Tyrosinase (Tyr) was incorporated to Langmuir films of arachidic acid (AA) and a bisphtalocyanine ($LuPc_2$) using the strategy of adsorption from the subphase. The addition of NaCl was a requirement for Tyr adsorption onto a bare interface and onto the films. A large expansion was observed in the surface pressure isotherm of the mixed monolayer indicating the protein incorporation. Platinum electrodes were modified with Tyr LB films as probed by FTIR and electrochemistry. The applicability of such films as biosensors is envisaged.

Biosensors make use of specific reactions between biological molecules to detect molecules of interest in several media [1]. In this field of research, the use of enzymes and enzymatic reactions is of outstanding importance and many authors report the immobilization of such materials in thin films [2, 3]. The material and the way with which enzymes are attached onto solid supports play a vital role in the enzyme activity preservation, and therefore on the biosensor efficiency and applicability. Thus, the first step in working with enzyme-based biosensors is the effective immobilization of the biomolecule preserving its functionality.

In this study we have used nanostructured Langmuir-Blodgett (LB) films as solid supports for the enzyme Tyrosinase (Tyr, *from mushroom, with activity of 5370 U/mg*). The enzyme was first incorporated to mixed Langmuir films of arachidic acid (AA), employed as matrix, and Lutetium bisphtalocyanine ($LuPc_2$), which was used to help as electron transfer mediator in further electrochemical analysis. A previous study at the interface showed that AA and $LuPc_2$ form almost completely miscible monolayers, with little excess of free energy being observed for mixtures with five different proportions. The incorporation of Tyrosinase from the buffered subphase was monitored through changes in the surface pressure. Apart from the water-soluble character of Tyrosinase, the protein could migrate to the interface with the addition of NaCl. A Gibbs monolayer was formed and after compression surface pressure values as high as 20 mN/m were achieved. The incorporation to the mixed (1:1 in mol) AA-$LuPc_2$ film was confirmed by a $\Delta\pi$ of ca. 3 mN/m observed before compression for an expanded monolayer. In addition, some expansion was observed for the surface pressure (π x A) isotherm (figure 1) pointing to the protein incorporation to the film. Platinum electrodes were modified with these Tyrosinase-containing mixed monolayers. Z-type LB films were deposited according to the transfer ratio values in figure 2. Moreover, the presence of the protein in the modified electrodes was demonstrated in electrochemical and FTIR measurements. Such films will be tested for the biosensing of antioxidant molecules.

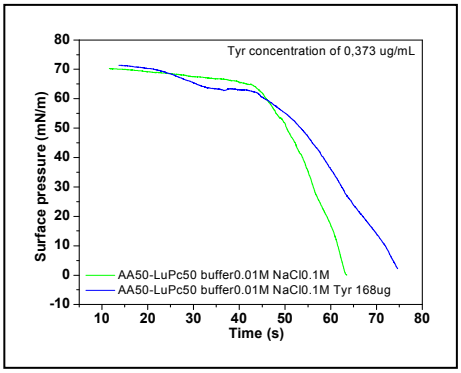

Figure 1: π x A isotherms for a mixed Langmuir film of AA-$LuPc_2$ on a buffered subphase and on a subphase containing 0.373 μg/mL Tyr

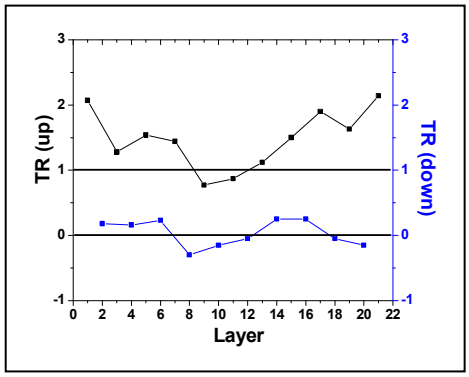

Figure 2: Transfer ratio values for the deposition of a mixed AA-$LuPc_2$-Tyr LB film over a Platinum electrode

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