Microscopy analysis of chitosan-peroxidase layer-by-layer films

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The immobilization of enzymes and other biomolecules has been exploited in producing biosensors, which requires suitable methods to produce ultrathin films capable of retaining the bioactivity. One of such techniques is the layer-by-layer (LbL) method based on the adsorption of alternated layers of oppositely charged species [1]. In this study we used the LbL technique to immobilize the enzyme horseradish peroxidase (HRP) on a matrix of the biocompatible polymer chitosan (Ch) and obtain a hydrogen peroxide sensor. The solid supports were alternately immersed in a solution of chitosan (1 g L^{-1}) and HRP (0.5 g L^{-1}), with seven bilayers of Ch/HRP being deposited with adsorption times of 10 min. for the chitosan layer, while for HRP this time varied from 30 to 6 minutes for the first and last bilayers, respectively. An appreciable enzymatic activity was measured, which was obtained indirectly with UV-visible spectroscopy. The film morphology investigated with optical and atomic force microscopies was affected by the measurements in which the LbL films were exposed to a solution containing hydrogen peroxide. Stripes were seen in the film, illustrated in the AFM image of Figure 1, which also applied to the microscopic scale studied with optical microscopy, but these stripes disappeared after the film was used as a biosensor This is in contrast to HRP co-immobilized with phospholipids in Langmuir-Blodgett (LB) films of an earlier study, where activity was preserved for long periods of time and several uses of the sensing unit. The molecular architecture and the scaffolding material for enzyme immobilization are thus crucial for the film morphological properties and activity of the enzymes.

Keywords: layer-by-layer, biosensor, microscopy analysis, morphological structure.

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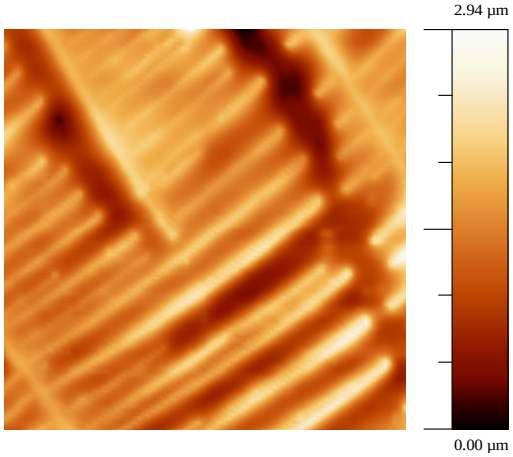

Figure 1: AFM of Ch-HRP LbL film before enzyme activity measurement.