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Characterization of Nanostructured Films to be Applied in Biosensors for Glucose and Sucrose

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Abstract – Nanostructured films obtained with the layer-by-layer technique of poly(allylamine) hydrochloride (PAH), glucose oxidase (GOx), and invertase (INV) have been used to produce biosensors for determining glucose and sucrose simultaneously. Optimized conditions of pH and concentration of the solutions were identified for building the PAH/GOx, PAH/INV and PAH/GOx/PAH/INV LbL films, which were characterized using UV-vis spectroscopy and electrochemistry.

Biosensors containing enzymes have been widely used in different areas due to their high sensitivity and selectivity [1,2]. Important among such biosensors are those to detect glucose and sucrose, not only for diagnosis of diabetes but also because sucrose is the major ingredient of foodstuffs and sweet drinks [3]. Here we report an alternative method for identifying two kinds of sugars simultaneously, where the sensing units were made with layer-by-Layer (LbL) films with immobilized enzymes. This builds upon previous work in our group, in which enzymes such as uricase, glucose oxidase (GOx) and phytase were adsorbed onto indium tin oxide (ITO) substrates coated with a layer of Prussian Blue (PB). Immobilized enzymes retain their activity in LbL films because the film-fabrication process is carried out under mild conditions, and entrained water remains in the film structure.

The glucose oxidase (GOx) and invertase (INV) enzymes were immobilized in alternating layers with poly(allylamine) hydrochloride (PAH). Using UV-vis spectroscopy (Figure 1a) the enzyme solutions and the LbL film growth were characterized. The linear growth is indicative that the same amount of enzyme has been adsorbed in each bilayer. Figure 1b shows amperometric data for the ITO/PB/$(PAH/GOx)_{10}$ system, with PB as an electrochemical mediator of oxygen peroxide H_2O_2 produced by oxidase enzymes. Electric current changes at 0.0 V potential *vs* saturated calomel electrode (SCE) were observed upon each addition of 100 µL of the 10 mM glucose solution. The film buildup was also investigated with fluorescence measurements, as shown in Figure 1c for a film containing the two enzymes in a single sample (PAH/GOx/PAH/INV). These films will be employed for a possible application on biosensors for detecting glucose and sucrose simultaneously in commercial beverages.

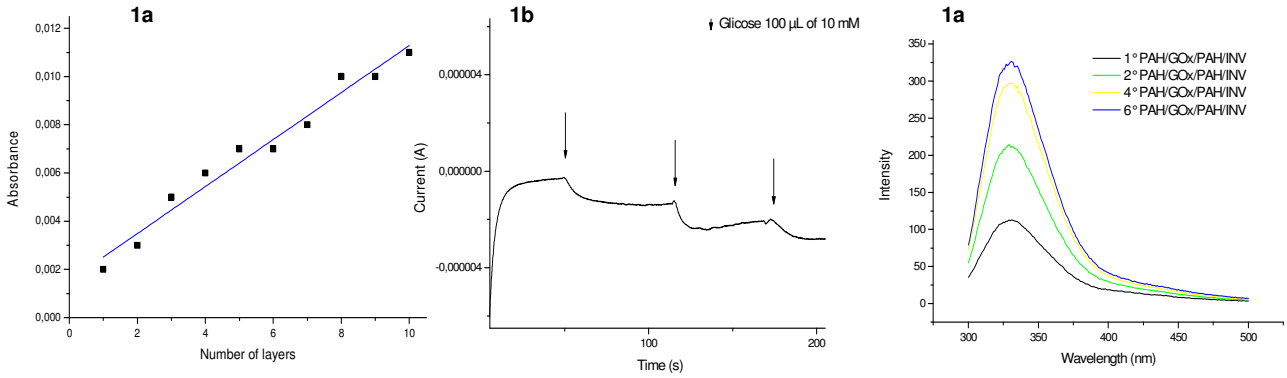

Figure 1: a) Linear growth of 10-bilayer PAH/GOx film. **b)** Amperometric response obtained at 0.0 V in a buffer solution at pH 6,3 with a 10-bilayer PAH/GOx deposited onto ITO/PB upon additions of glucose. **c)** Fluorescence bands at 330 nm of PAH/GOx/PAH/INV film with wavelength excitation at 280 nm.

References

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