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# Low-cost biosensors based on tyrosinase for analyzing phenols in aqueous media

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**Abstract** – We fabricated biosensors based on the use of crude extract from avocado fruit as an enzymatic source of tyrosinase and compared their response to those obtained with commercially, purified enzyme. The amperometric biosensors were fabricated by chemically immobilizing the enzymes on self assembled monolayers of 3-mercaptopropionic acid deposited on Au electrodes. Using chronoamperometric characterization we obtained pH and concentration by using sequential injections of the analyte solutions into the flow injection analysis system, which were connected to the electrodes, for both biosensors. Similar results were obtained for both biosensors indicating efficient biosensors can be produced from the enzymatic crude extract.

In order to fabricate a low cost tyrosinase biosensor, we used the enzyme in its natural source as crude extract of avocado fruit (*Persea americana*) [1], since commercially, purified enzyme are relatively expensive and not promptly obtained such as fresh vegetables and fruits. The enzymes were chemically immobilized on self-assembled monolayers (SAMs) of 3-mercaptopropionic acid (MPA) by using pentraflurphenol (PFP) and carbodiimide (EDC) on Au electrodes. The Au/SAMs electrodes were prepared by immersing the Au electrodes in a MPA ethanolic solution. The electrochemical measurements were performed by using an electrochemical cell with three electrodes, Au/MPA electrode as the working electrode, a Pt disk as the counter electrode and Ag quasi-electrode as the reference. The electrochemical cell was connected to a flow injection analysis (FIA) system for measuring the analytical response of the modified electrodes. The values of pH and concentration were obtained after injecting 200 µL of phenolic compounds at different concentrations from the FIA system. The voltammetric responses of the modified electrodes were obtained first to detect the values of the maximum oxidation potential. The enzymatic activity was determined by measuring the variation of absorbance for quinone produced during the oxidation catalytic reaction of phenols, which was catalyzed by tyrosinase.

The enzymatic activities were 259 and 479 units of active enzyme per milliliter (u $mL^{-1}$) for crude extract and purified enzyme solutions, respectively. For the biosensors prepared from purified enzyme and crude extract, respectively, we obtained the values of peak potentials for oxidation of cathecol , optimum pH and detection limit as 418 mV and 365 mV; pH 6,8 and pH 7,0; 6,65 mmol $L^{-1}$ and 4,65 mmol $L^{-1}$. These values indicate that miniaturized, efficient, low cost biosensors can be fabricated based on tyrosine from avocado's fruit crude extract. The main results obtained are shown in Figures 1 and 2.

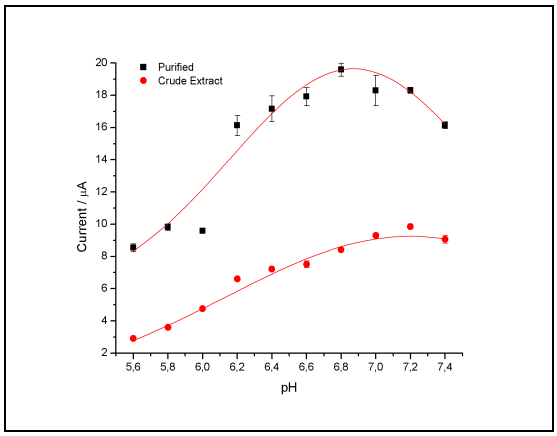


**Figure 1:** Optimum values of pH for purified enzyme and crude extract biosensors.

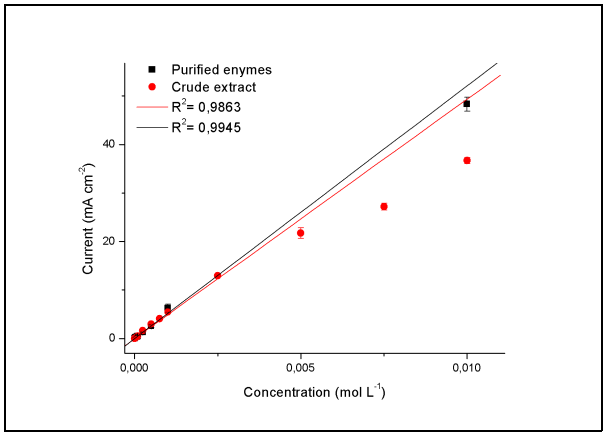


**Figure 2:** Calibration curves for purified enzyme and crude extract biosensors.