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New amperometric biosensor for determination of heavy metal ions based on the enzymatic inhibition of HRP immobilized on ferrocenyl polycyclosiloxane/Gold Nanoparticles modified electrode

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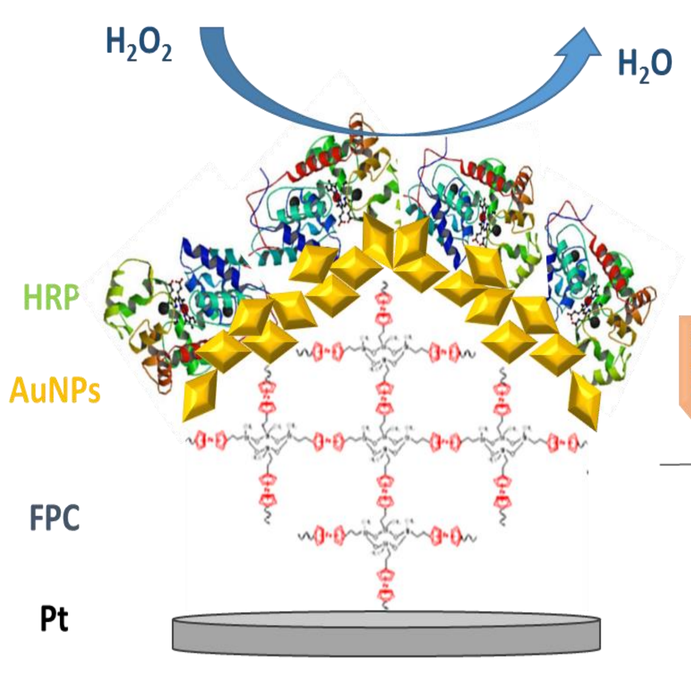
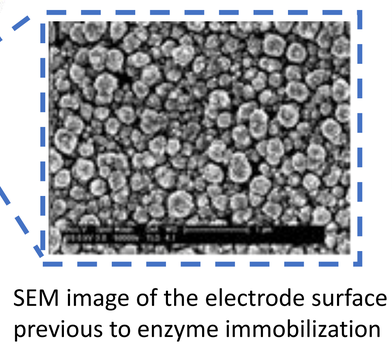
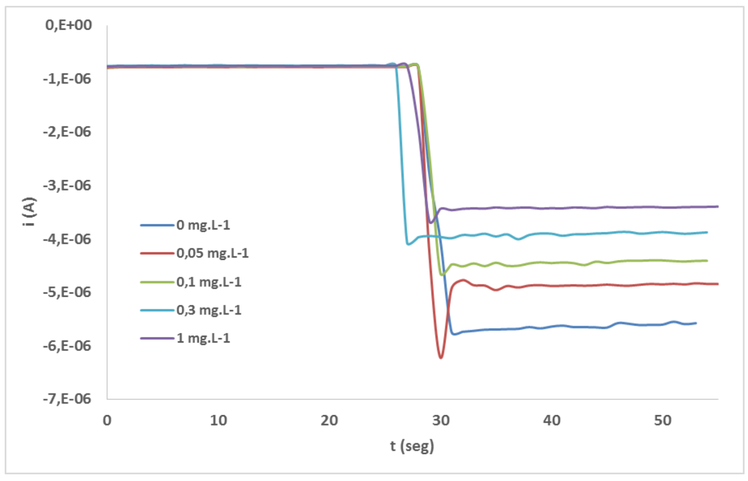
Heavy metals such as lead, copper, cadmium and mercury are non-degradable, cannot be detoxified biologically and can accumulate in the biosphere and transfer to the alimentary chain, thereby giving arise to potential serious health consequences for human beings, animals and plants [1]. Lead and cooper ions reach the environment from sources as PVC pipes in sanitation, recycled PVC lead paints, lead batteries, fertilizers, tanning, and photovoltaic cells [2]. The enzyme inhibition caused by heavy metals has provided a new way to develop inhibition-based biosensors [3]. In this sense, horseradish peroxidase (HRP) is one of more used enzymes because its low cost, availability and easy immobilization. The AuNPs present a high biocompatibility with the enzyme Horseradish peroxidase (HRP) maintaining their bioactivity [4] and improve the sensitivity of biosensors. In this poster, we present the first results obtained with an amperometric hydrogen peroxide (H_2O_2) biosensor developed by covalent immobilizing of HRP onto AuNPs electrodeposited from a ferrocenyl polycyclosiloxane (FPC) film, also electrodeposited on a glassy carbon electrode [5]. The biosensor show direct electrochemistry with the HRP and now is beening successfully applied to the indirect determination of Pb^{2+} and Cu^{2+} based on the inhibition of the enzyme.

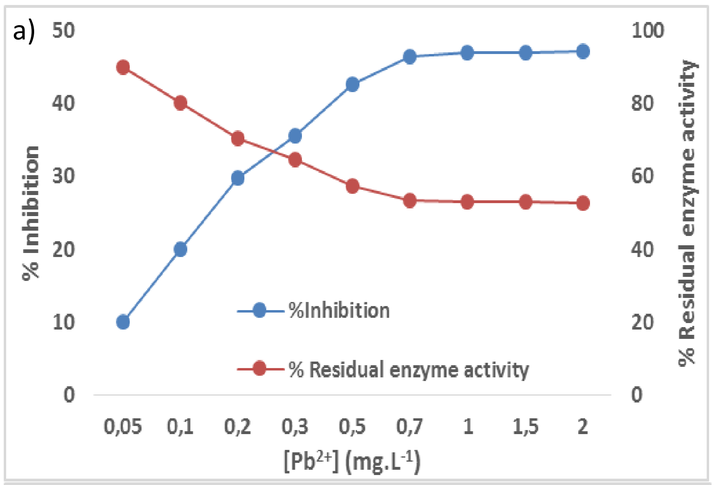


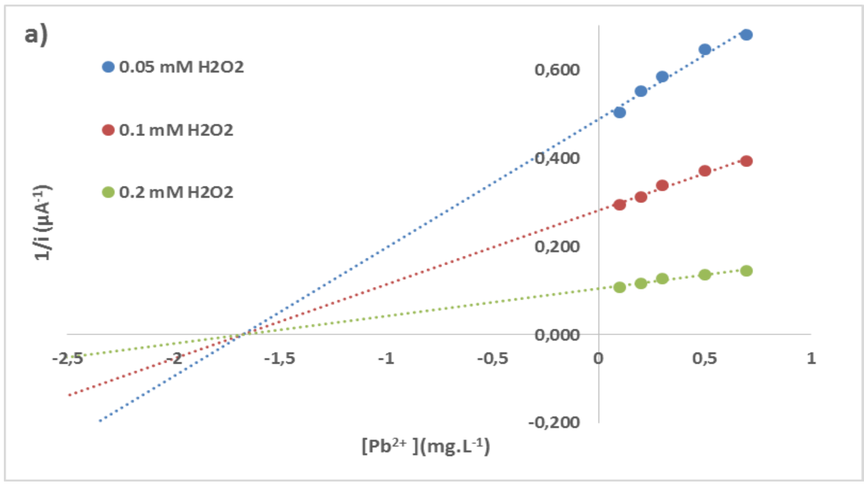


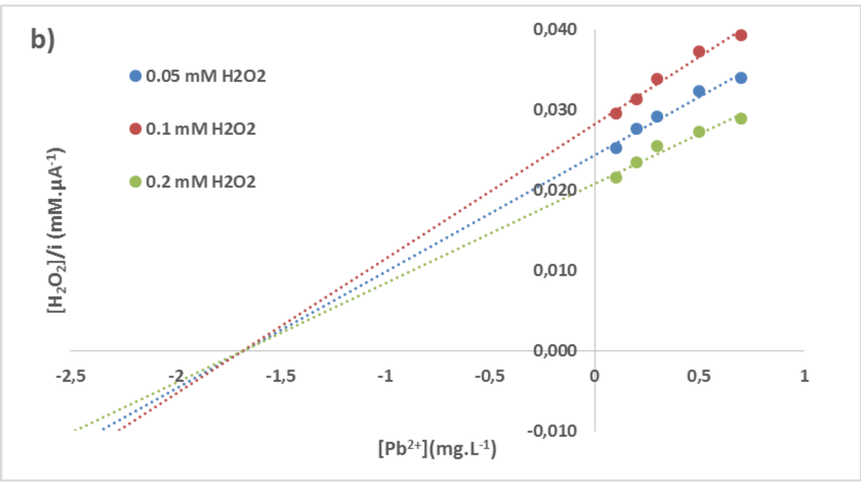
SEM image of the electrode surface previous to enzyme immobilization



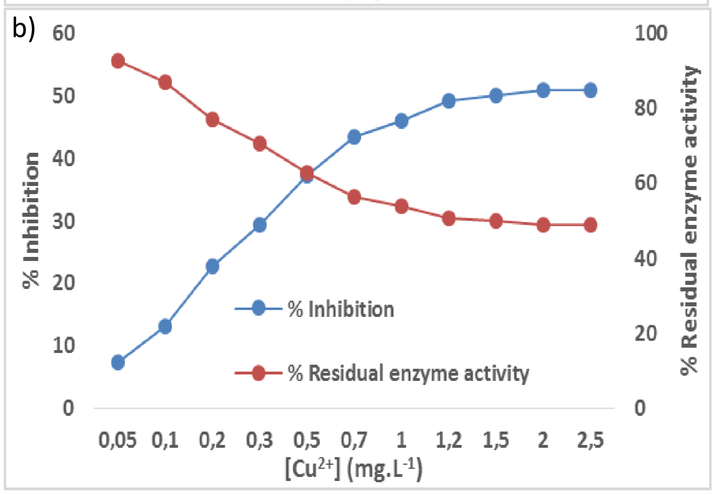
Scheme of biosensor and their response in absence and presence of lead, at -0.3 V in 0.01M Phosphate buffer pH 7 0.1M $NaClO_4$

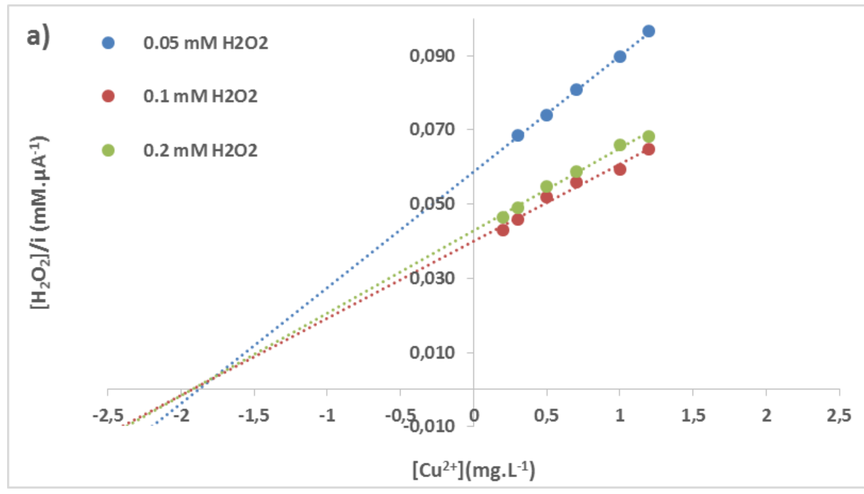


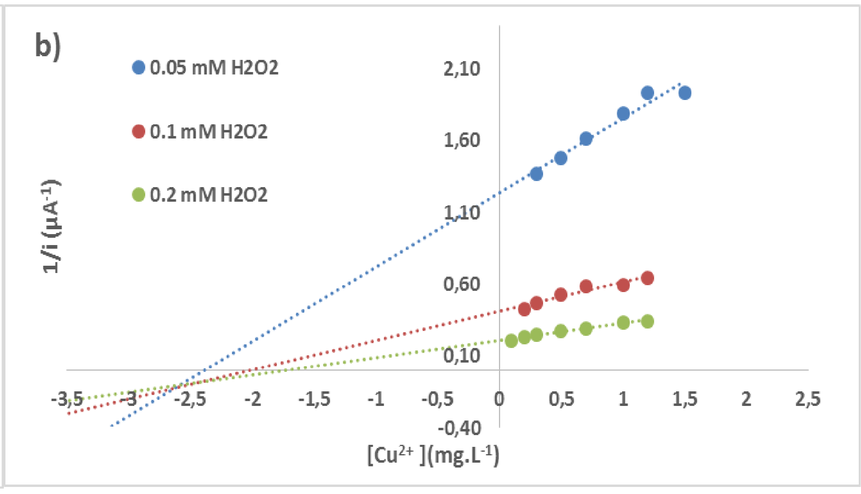




Dixon a) and Cornish-Bowen b) plots of the effect of different Pb^{2+} concentration on HRP







Dose-depent enzyme inhibition and residual enzyme activity towards HRP-catalyzed for the heavy metal ions Pb2+(a) and Cu^{2+} (b)in presence of H_2O_2 0.1 mM. Error bar ± SD and n=3

Dixon a) and Cornish-Bowen b) plots of the effect of different Cu^{2+} concentration on HRP

RESULTS

Heavy metal ion	Concentration range ($mg.L^{-1}$)	Linear range ($mg.L^{-1}$)	K_i ($mg.L^{-1}$)	K'_i ($mg.L^{-1}$)
Pb^{2+}	0.05 – 2.5	0.05 – 0.7	1.68	1.68
Cu^{2+}	0.05 – 2.5	0.05 – 1.2	1.77	2.68

Conclusions: According to Dixon [6], the Pb^{2+} shows a behavior of non - competitive inhibition, with inhibition constant K_i equal to the dissociation constant of the enzyme-inhibition-substrate (EIS) K'_i, in agreement with Bowed [7]. The Pb^{2+} reduces the activity of the enzyme and attaches to it even if the enzyme has already bond the substrate. Meanwhile the Cu^{2+} shows a mixed inhibition with $K'_i > K_i$, and it may bind to the enzyme whether or not it has already bond the substrate.

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