ENHANCED FUNCTIONALITY OF PEROXIDASES BY ITS IMMOBILIZATION ON CdS NANOPARTICLES AND THEIR ACTIVATION THROUGH UV LIGHT.

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Introduction. Peroxidases (POs) are versatile enzymes that catalyze the oxidation of a wide variety of compounds of commercial and environmental interest. In spite of their attractive characteristics, their commercial and industrial application is still negligible because of their low operational stability. Several strategies for improving this aspect have been successfully explored, including enzymatic immobilization (1). Another parameter affecting their usage is the requirement of hydrogen peroxide as cosubstrate, which can inhibit enzymatic activity. To overcome this, POs have been activated through UV irradiation of semiconductive nanoparticles in solution (2). However, this approach has not been studied when both nanoparticles and POs are immobilized in a solid surface (3). This is the objective of the present work.

Methods. CdS nanoparticles were synthesized using the reverse micelle method (4). Silica surfaces were cleaned in a plasma chamber and silanized with vaporized 3 – aminopropyltriethoxysilane (APTES). Then they were functionalized with glutaraldehyde as a spacer. Afterwards, CdS nanoparticles were adsorbed on these surfaces, followed by chloroperoxidase (CPO, from *Caldariomyces fumago*) immobilization. The adsorption kinetics were measured in real – time using a quartz crystal microbalance (QCM) (5). Enzymatic activity was measured by injecting the substrate (Amplex Red) into the solution in contact with the surface (Phosphate buffer, 60 mM, pH=6, 7 ºC), then irradiating with 365 nm UV light for 50 minutes (in cycles of 5 min irradiated and 2 min in the dark). After this, product (resorufin) concentration was measured through UV – Vis spectroscopy. Finally, this concentration was compared with that obtained through hydrogen peroxide activation of CdS-CPO system in solution.

Results. Functionalization of silica surfaces with APTES and glutaraldehyde was successfully completed (results not shown). CdS nanoparticles formed a first layer on the surface, and then continued being adsorbed as indicated by the second frequency shift (Fig.1).

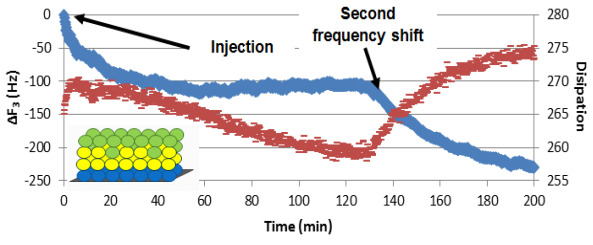

Fig.1 Adsorption kinetics of CdS nanoparticles on previously functionalized silica surfaces.

CPO adsorbed quickly on the CdS surface, and formed a layer which showed stability to posterior rinsings (Fig.2). Finally in Table 1 we show a comparison between the CdS-CPO biocatalyst system's performance in solution and immobilized on silica surfaces, with the latter showing 4.63 times more activity than the first.

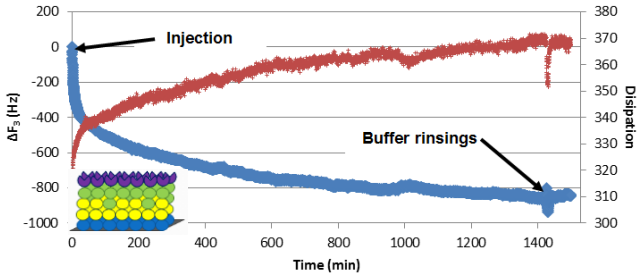

Fig.2 Adsorption kinetics of CPO on silica surfaces with CdS nanoparticles previously adsorbed.

Table 1. Comparison of oxidized substrate percentage (OSP, relative to H_2O_2 oxidation) between the biocatalyst system in solution and immobilized on silica surfaces.

	In solution	With immobilized system
OSP % = 49.093*Abs + 2.012	9.13	8.39
OSP Minus control (Abs 0.098)	4.17	3.58
[CPO] present	2 nmol	0.3701 nmol
OSP / [CPO] present	2.09	9.67

Conclusions. The CdS-CPO biocatalyst system was successfully immobilized in silica surfaces, displaying good stability to buffer rinsings and more activity than the system in solution. However, most of the material desorbed from the surface during rinsing after irradiation. That is the focus of our current research efforts.

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