SENSITIVITY INCREASE OF ENZIMATIC QCM SENSOR FOR TRYPSIN ACTIVITY DETERMINATION BY NANOPARICLES APLICATION

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Introduction. Trypsin (EC 3.4.21.4) is a protein degrading enzyme of the group of the serine proteases having many applications: in food and beverage industries for protein hydrolysates production, in cold stabilization of beer etc. It is employed in biochemistry for protein identification through peptide sequencing techniques [1]. The activity of trypsin serves as a reliable diagnostic test of pancreatic function and its alteration [2].
The industrial, biotechnological, and biochemical importance of trypsin, as well as its clinical significance make important the development of methods for quantification of its activity. Many methods already have been developed mainly radioimmunoassay-based and spectrophotometric techniques [3]. Methods based on measurement of the change of temperature, pressure [4] and humidity [5] due to the action of trypsin on gelatin films were recently developed as well. Unfortunately the known sensors and methods are very complicated and slow.
A new approach for trypsin activity determination based on QCM application allowing significant LOD improvement and sensitivity increase is the subject of this work. The main point of this approach is the application of Ag nanoparticles loaded gelatin, deposited on the QCM crystal surface, serving as trypsin substrate. During the substrate enzymatic degradation by trypsin, the heavier nanoparticles also leave it together with the substrate degradation products, provoking thus a greater mass change, compared with the no loaded substrate. As a result the QCM frequency response depending on the total QCM crystal mass increases, improving thus the LOD and the sensitivity of the trypsin determination.

Methods. The Ag nanoparticles were electro-generated directly in the water used for the gelatin solution preparation, which was deposited on the QCM crystal by spin coating method. Trypsin solutions with different concentrations obtained by dilution having different enzim activity were employed for two calibration plots building: with the application of no loaded and Ag nanoparticles loaded gelatin as a substrate. The LOD was determined using very diluted solutions of trypsine.

Results. The QCM response consists of an initial frequency decrease due to the mass increase attributed to the enzymes adsorption on the gelatin layer. The enzyme substrate degradation and the products departure to the solution provoke a decrease of the total crystal mass which results in a frequency augmentation which represents the QCM response. Finally, the complete substrate layer degradation causes a plateau formation on the QCM frequency-time curve.
The calibration plot shown in Fig. 1 correspond to a trypsin range from 0.01 µg mL-1 up to 1000 µg mL^{-1} employing a bulk (curve a) and nanoparticles loaded (curve b) gelatin as a substrate.

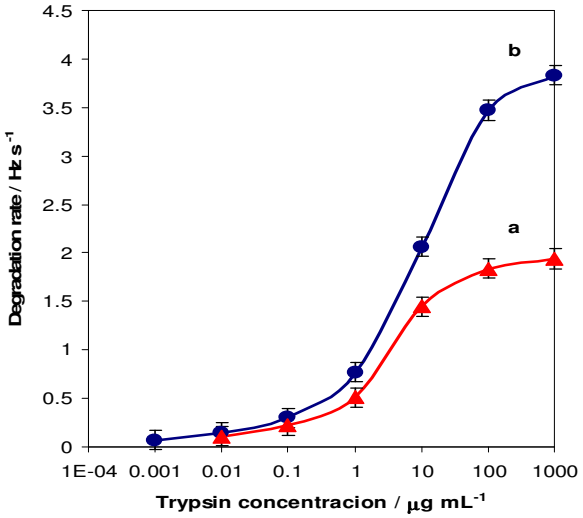

Fig.1 Calibration plots for trypsin activity evaluation

The nanoparticles application resulted in a 10-fold detection limit improvement down to 0.0001 µg mL^{-1} instead of from 0.001 µg mL^{-1}, obtained with no loaded substrate

Conclusions. A simple and efficient QCM based approach for trypsin activity determination was proposed and tested. Ag nanoparticles loaded substrate application resulted in a 10-fold detection limit improvement compared with no loaded substrate.

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