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Thermolysin Entrapment in a Gold Nanoparticles/Polymer Composite: Construction of an Efficient Biosensor for Ochratoxin a Detection

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Abstract : An original method has been successfully developed for the immobilization of thermolysin onto gold interdigitated electrodes for the detection of ochratoxin A (OTA) in olive oil samples. A mix of polyvinyl alcohol (PVA), polyethylenimine (PEI) and gold nanoparticles (AuNPs) was used. Cross-linking sensors chip was made by using a saturated glutaraldehyde (GA) vapor atmosphere in order to render the two polymers water stable. Performance of AuNPs/ (PVA/PEI) modified electrode was compared to a traditional immobilized enzymatic method using bovine serum albumin (BSA). Atomic force microscopy (AFM) experiments were employed to provide a useful insight into the structure and morphology of the immobilized thermolysin composite membranes. The enzyme immobilization method influence the topography and the texture of the deposited layer. Biosensors optimization and analytical characteristics properties were studied. Under optimal conditions AuNPs/ (PVA/PEI) modified electrode showed a higher increment in sensitivity. A 700 enhancement factor could be achieved with a detection limit of 1 nM. The newly designed OTA biosensors showed a long-term stability and good reproducibility. The relevance of the method was evaluated using commercial doped olive oil samples. No pretreatment of the sample was needed for testing and no matrix effect was observed. Recovery values were close to 100% demonstrating the suitability of the proposed method for OTA screening in olive oil.

Keywords : thermolysin, A. ochratoxin , polyvinyl alcohol, polyethylenimine, gold nanoparticles, olive oil
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