AMPEROMETRIC INMUNOSENSORS FOR OCHRATOXIN A (OTA) BASED ON SCREEN PRINTED ELECTRODES (SPCES) NANOSTRUCTURED WITH GOLD NANOPARTICLES

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Ochratoxin A (OTA) is a mycotoxin with nephrotoxic, teratogenic, carcinogenic and immunotoxic activity in human and animals. OTA occurs in several foodstuffs such as cereals, coffee beans, nuts and cocoa. There are very strict mycotoxin regulations all over the world [1].

Chromatographic analytical methods available for the analysis of mycotoxins in food , beverages,and foodstuffs are usually validated by AOAC International Official Methods of Analysis [2].

The direct voltammetric analysis of OTA is possible [3], but the oxidation of this molecule needs a very high potential (+1.5 V). Besides, sensitivity is low, and then the direct amperometric determination of OTA is not suitable at the level of low ppb required. For these reasons the use of immobilized antibodies is more convenient when amperometric transducer are used

We are developing a rapid and sensible OTA immunosensors using advantages of the immunochemical assay and the nanostructured screen-printed technology. We have studied the influence of gold nanoparticles (AuNP, 20 nm. diameter) directly immobilized on screen printed graphite electrodes (SPCEs) made in our lab in order to increase the analytical signal obtaining higher voltammetric currents in the detection step. The Quartz Crystal Microbalance (QCM) was used to demonstrated that the nanofunctionalization process with AuNP increases the real sensoring surface of the quartz crystal device

The use of AuNP as versatile and efficient substrates for the immobilization of antibody or antigen showed not only enhance the amount of antibodies or antigens immobilized on the electrode sensing surface, also preserve the activity of the immobilized biomolecules. Nanometer-size (20nm) AuNPs exhibit excellent catalytic activity and these AuNPs have a relative high surface area-to-volume ratio.

We used indirect immunoassays in a competitive way by immobilizing OTA-BSA (ochratoxin A with serum albumin bovine) conjugate on the SPCEs and using alkaline phosphatase (AP) or horse-radish peroxidise (HRP) as transduction enzymes labelled to secondary anti-IgG antibodies to generate the amperometric signal. Also we used bovine serum albumin (BSA) for blocking and avoiding unspecific adsorption.

The electrochemical substrate for generating the amperometric signal was 1-naphtil phosphate for AP and hydroquinone/H_2O_2 for HRP. The enzymatic product of both reactions was detected by differential pulse voltammetry (DPV).

The designed immunosensors were compared with spectrophotometric ELISAs (enzyme-linked immunosorbent analysis) using 4-nitronaphtyl phosphate as enzymatic substrate and

measuring the UV-VIS molecular absorbance of 1-nitronaphthol at a wavelength of 405 nm obtaining good validation analytical results.

Besides, the analytical results were validated with official methods based on HPLC with fluorescence detection. Regeneration of the immunosensor surfaces has been studied. And the SPCEs with AuNPs were observed by Scanning Electron Microscopy (SEM) in order to characterize their nanostructured surface. In conclusion, *SPCEs* inmunosensors for OTA allow the quick and specific determination of this mycotoxin at the ppb levels. The formation of a layer of nanostructured particles (AuNPs) increased the sensitivity of the transduction process in this new OTA amperometric immunosensors.

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