An electrochemical competitive biosensor for deoxynivalenol based on paramagnetic microparticle beads functionalized with protein A

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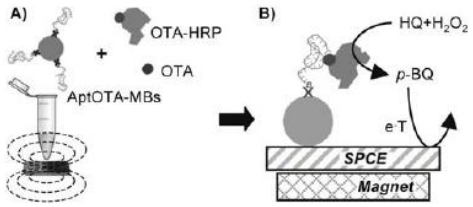
The work done so far have been based on the design of an electrochemical competitive aptamer-based biosensor for ochratoxin A (OTA) [1,2]

OTA is one of the most important mycotoxin contaminants of foods, particularly cereals grains (corn, barley, wheat and rye) and cereal products, with strict low regulatory levels (of ppb) in many countries worldwide. The highest OTA concentration in unprocessed cereals and cereal products permissible by the European Community is 3–5 μg kg^{-1} (Commission Regulation No. 1881/2006).

Paramagnetic microparticle beads (MBs) were functionalized with an aptamer specific to OTA, and were allowed to compete with a solution of the mycotoxin conjugated to the enzyme horseradish peroxidase (OTA–HRP) and free OTA. After separation and washing steps helped with magnetic separations, the modified MBs were localized on disposable screen-printed carbon electrodes (SPCEs) under a magnetic field, and the product of the enzymatic reaction with the substrate was detected with differential-pulse voltammetry. In addition to magnetic separation assays, other competitive schemes were preliminary tested, optimized and compared. From all these kind of biosensors for OTA, electrochemical inmunosensors have the advantages of simplicity and sensitivity.

The magnetic aptasensor showed a linear response to OTA in the range 0.78 to 8.74 ng mL^{-1} and a limit of detection of 0.07±0.01 ng mL^{-1}, and was accurately applied to extracts of certified and spiked wheat samples with an RSD lower than about 8% [3].



In the present experimental work is to undertake the design of an immunosensor for the detection of deoxynivalenol (DON) in cereals, following the steps carried out for the OTA. The biosensor will be a direct immunosensor (immobilized biorecognition element) and competitive (competitive reaction between the DON and DON-HRP) and mode of detection is performed by a electrochemical technique.

Deoxynivalenol is a mycotoxin produced by Fusarium fungus, one of the most abundant fungi, which are abundant in certain cereals such as wheat, corn, barley, oats, and rye and their processed grains such as malt, beer or bread. DON inhibits the synthesis of DNA and RNA and protein ribosomes. This toxin has a hemolytic effect on erythrocytes. In high doses, DON causes vomiting, and if the concentration taken in the diet is lower growth and reduced food consumption (anorexia).

Maximum permissible concentrations of deoxynivalenol for different foods, are between 200 and 1750 μg/Kg, and the tolerable daily intake is 1 μg/Kg body weight.

The immobilization of anti-DON on paramagnetic beads functionalized with protein A (MBs) is based on the antiDON and protein A interaction. The sample of the antiDON-MBs is mixed in an eppendorf vial with a previously mixed solution containing DON and the conjugate DON–HRP (a fixed concentration).
After of incubating at room temperature, the global reaction is: MBs-protein A+anti-DON + DON + DON-HRP.
A sample of the global reaction is dropped onto the surface of the SPCE. For the specific location of the beads close to/on the electrode surface, a magnetic block is placed just at the bottom of the SPCEs. Then hydroquinone and hydrogen peroxide are deposited in this order onto the SPCE strip, and after 10 minutes at room temperature the enzymatic product (*p*-BQ) is determined using chronoamperometry.

References

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