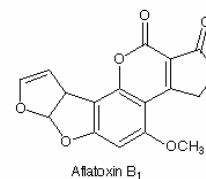


LABEL-FREE IMMUNOSENSOR FOR Aflatoxin B

using OPTICAL WAVEGUIDE LIGHTMODE SPECTROSCOPY (OWLS) detection

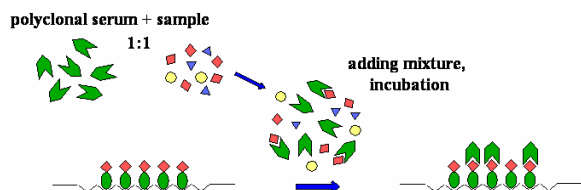
Abstract

Aflatoxins are toxic metabolites produced by *Aspergillus* species (mainly *A. Flavus*, *A. parasiticus*, and *A. nomius*) and can be present in a wide range of food and feed commodities. Because of the persistence of Aflatoxins in the food chain, exposure to the compound is a potential human health hazard. This has prompted adoption of regulatory limits in several countries which, in turn, implies the development of suitable validated and official analytical methods and rapid screening tests for cost-effective food control on a large scale. OWLS offered a highly sensitive label-free method to develop immobilized antigen- BSA conjugate based competitive immunosensor.



Application of OWLS sensors

as competitive immunosensor for the detection of Aflatoxin B₁



Surface Chemistry of OWLS sensors

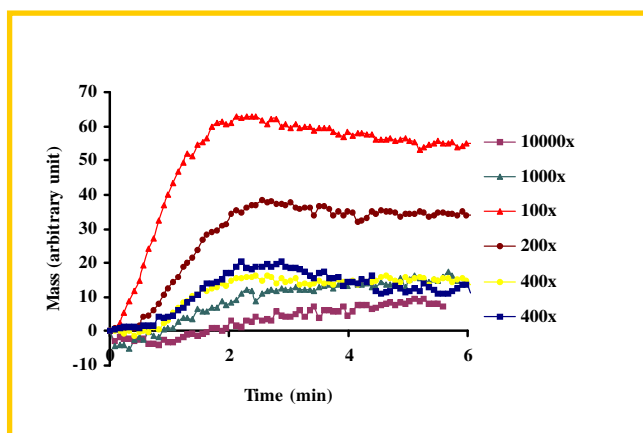
- Amino functionalisation of waveguide surface by 3-aminopropyltriethoxysilane
- Immobilisation of Aflatoxin-BSA conjugate (10 µg/ml) on the OWLS sensor surface by glutaraldehyde (2,5%).

Competitive assay format

Standards and samples were mixed with antibody, incubated for a certain period and the mixture was injected into the OWLS system. During determination, toxin present in the sample competes for binding of the antibody to the toxin conjugate immobilized on the sensor surface. Upon incubation, only antibodies remaining in free form in the sample mixture bind to the antigens immobilized on the sensor surface. Thus, the amount of antibodies bound to the surface of the chip was inversely proportional to the Aflatoxin B₁ content in the samples.

Optimization of antiserum dilution

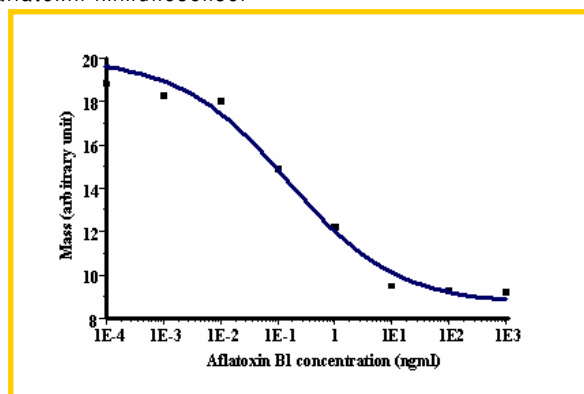
The antibody concentration employed is one of the parameters of key importance, because increasing toxin concentrations in the sample result in larger decreases in the assay signal.



Selection of optimal antibody dilution for competitive measurement of Aflatoxin B₁

Standard inhibition curve

of the immobilized antigen conjugate based competitive aflatoxin immunosensor

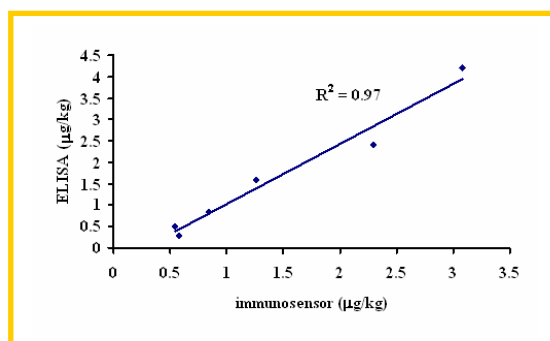


The sensitive detection range of the competitive detection method was between 0.5-10 ng ml⁻¹ when measuring Aflatoxin B₁.

Sample measurement

Extraction procedure

- Weight 1 g of sample and add 10 mL of acetonitrile/water (6:4; v/v) mixture. (Complete cereal grains should be grinded)
- Stirred for 5 min
- Decante and filtrate using a UF membrane with 100.000 NMWL.
- (5 min centrifugation at 5000 rpm)
- Dilute the filtrate at 100 fold dilution with 100 fold dilution of acetonitrile/water mixture in TRIS buffer



Comparing the results from OWLS measurement to that obtained by ELISA method, the regression coefficient (R^2) for Aflatoxin was calculated 0.97. It can be stated that the competitive immunosensor based on OWLS detection could be suitable for the quick determination of Aflatoxin level in grain samples.

References

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2. Adányi N., Levkovets I.A., Rodriguez-Gil S., Ronald A., Váradi M. Szendrő I. (2006): Development of immunosensor based on OWLS technique for determining Aflatoxin B₁ and Ochratoxin A • *Biosensors and Bioelectronics*, 22(6) 797-802.
3. www.owls-sensors.com